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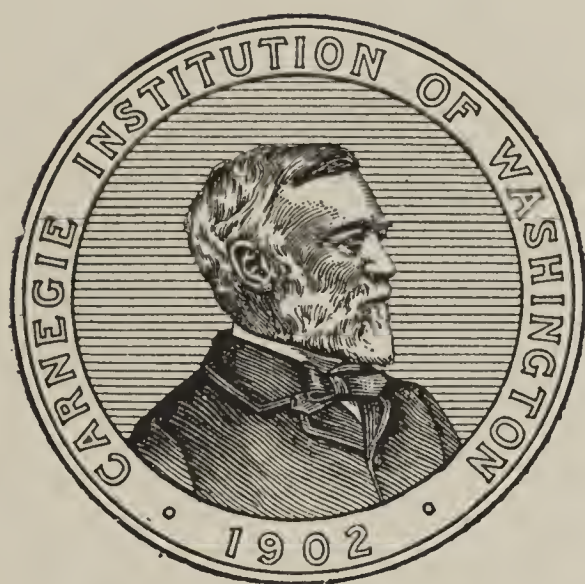
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THE HYDROSTATIC-PNEUMATIC SYSTEM OF CERTAIN TREES: MOVEMENTS OF LIQUIDS AND GASES

BY

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THE HYDROSTATIC-PNEUMATIC SYSTEM OF CERTAIN TREES: MOVEMENTS OF LIQUIDS AND GASES

THE LIQUID AND GAS SYSTEMS OF TREES

The hydrostatic systems of plants, especially trees, in which the photosynthetic and transpiratory organs are widely separated from the roots, through which water, electrolytes, and non-electrolytes in solution enter and pass upward into the shoot, has enlisted the keenest attention of plant physiologists for two and a half centuries. The amount of water transpired by the leaves and the energy required for lifting such large quantities as is transpired by the crown of a large tree is in some cases enormous. Since the publication of Stephen Hales' book on "Vegetable Statics or Experiments on the Sap of Vegetables" in 1727, in which he outlined experiments on sap-flow, a voluminous literature has arisen dealing with the different theories offered in explanation of the ascent of sap.

The system of liquids in plants, more particularly those with woody perennial stems, may be taken to include columns which are continuous from the roots to the leaves. Beginning with the layer of water coating the soil particles, there is, in the plant, a continuity of liquid through the root-hairs, cortex, and endodermis of the root, and through the conducting tracts of root and stem to their ultimate ramifications in the leaves. Water in the non-living elements of foliar organs, such as veins, is directly continuous with the vacuolar contents of the living cells of the leaf-parenchyma through their walls and protoplasmic layers. Some of these cells adjoin intercellular spaces so that part of their external surfaces are exposed to the intercellular cavities, which communicate with the external atmosphere through the stomata. The evaporation of water from the walls of these cells adjoining intercellular spaces tends to dry them out. The menisci formed by the water in the submicroscopical cavities in the exposed walls constitute the functional terminals of the water system, since it is from these curved surfaces that water particles are set free as vapor, resulting in an incipient drying of the exposed walls. The loss of water vapor results in a deformation of the menisci, and the resulting increased tension abstracts water particles from the contents of the cells which the walls enclose. Therefore, transpiration and the resulting successive deformation of the menisci in exposed walls of parenchymatous cells adjoining intercellular spaces eventu-

ally sets up a pull on the water in the conducting elements of the veins, which is in turn transmitted through the continuous water-columns of the stem and root, thus moving it upward through the stem to the leaves. Furthermore, on account of the high osmotic pressure of the cells of the leaves, owing to water being abstracted from them by transpiration, water will tend to pass into them from neighboring cells, which contain relatively more water. In turn, those cells lying next a vein eventually draw water from the water-containing elements of the vein. While the greater part of the water entering the root goes to the leaves, it should be remembered that the conducting tracts are in contact with vertically and radially arranged sheets of living cells through a large part of their length and also with masses of wood parenchyma. Some liquid is withdrawn from the conducting tracts by the osmotic action of these living elements of the medullary rays and wood parenchyma, in which the sap is almost always a more concentrated solution than is the upwardly moving liquid in the vessels and tracheids.

The cohesion theory of Dixon of the ascent of sap, especially in trees, is based upon the fact that water in the conducting tracts is brought into a state of tension, and that it is able to withstand considerable longitudinal pull without being broken, and upon the fact that the water exists in the conducting tracts in the form of an unbroken meshwork, even through their end and side walls, being uninterrupted by air-bubbles. The threads of water have a tensile strength with a breaking strain of over 300 atmospheres, and the pull which may be exerted upon them by the transpiring cells may be more than 200 atmospheres.

The effect of the presence of air as bubbles in vessels and tracheids containing some liquid has been a much-debated topic. At one time the presence of these bubbles, lessening the total weight of a theoretical column of water, was supposed to facilitate the ascent of sap in stems containing these "Jaminian chains." It seems now to be well-nigh universally conceded that the presence of a gas-bubble in a vessel or a tracheid at once eliminates that element from the conducting system. The condition and composition of the gaseous mixture which fills woody elements when vacated by the water of the transpiration stream, and the interaction of these gases and liquids, have been the object of special attention in the experiments, the results of which are given in the following pages.

Observations of the varying differential tensions in stems appear to have been made originally by Hofmeister in 1859, and contributions by Sachs and Millardet followed shortly. Further examination of this matter was made by Kraus¹ in 1866; and a decade later in con-

¹ Kraus, G. Die Gewebespannung des Stammes und ihre Folgen. *Botan. Ztg.*, 25: 105-119, 121-126, 129-132, 137-142. 1867.

nection with measurements of the relative distribution of water in curving stems and roots he concluded¹ that the increase which took place in stems at night resulted from the swelling of the "Rinde" alone. He saw not the slightest alteration in the diameter of the woody cylinder. From this it follows that he is not to be credited with the discovery of the principal facts in the daily alteration in thickness of woody stems. Shortly following an announcement to the above effect Dr. P. Kaiser,² a student of Kraus, made hourly measurements of the thickness of a number of trees. These measurements were obtained by the use of calipers of accuracy to determine the distance between two smooth places on the bark on opposite flanks of the tree. A somewhat more extended study by Kraus,³ to which reference is ordinarily made as the first contribution on this subject, was published in 1881. Hall⁴ measured changes in the diameter of a number of trees 1885-1890.

The identification of the daily alterations in diameter of a tree-trunk with swelling and shrinking of the woody cylinder, although hinted at by Dixon, was first clearly shown by MacDougal⁵ in the summer of 1920. Dendrographic records were made of a trunk of the Monterey pine (*Pinus radiata*) from bearings made on the wood completed the second season before (that is, in the summer of 1920 the wood formed early in that year, in the previous year, and in 1918 was removed and the bearings made on the wood laid down in 1917). The daily variation which amounted to 1 part in about 2,000 in early summer fell to 1 part in about 9,000 late in the summer. The swelling and shrinking was attributed to changes in the wood and to the seasonal lessening of transpiration, as has been described elsewhere. Variations in diameter were found to depend directly upon the depletion and repletion of the water supply in the wood. In a later publication⁶ the effect of increased tension in the water upon the diameter of the conducting elements was noted. Many writers noting the cessation of elongation of growing stems, or the not infrequent decrease in length during the daytime, ascribed this action to the retarding effects of light.

In connection with some studies on the ascent of sap, as the result

¹ Kraus, G. Die Vertheilung und Bedeutung des Wassers bei Wachstums- und Spannungsvorgängen in der Pflanze. Botan. Ztg., 35: 595-597. 1877.

² Kaiser, P. Ueber die tägliche Periodicität der Dickendimensionen der Baumstämme. Inaug. Diss. Halle, 38 pp. 1879.

³ Kraus, G. Die tägliche Schwellungsperiode der Pflanzen. Abhandl. d. Naturf.-Gesell. Halle, 15. 1881.

⁴ Hall, C. E. Notes on tree measurements, made monthly at San Jorge, Uruguay, from Jan. 12, 1885, to Jan. 12, 1890. Trans. Bot. Soc. Edinburgh, 18: 456-468. 1891.

⁵ MacDougal, D. T. Growth in trees. Carnegie Inst. Wash. Pub. No. 307 (especially pp. 39-41). 1921.

⁶ MacDougal, D. T., and Forrest Shreve. Growth in trees and massive organs of plants. Carnegie Inst. Wash. Pub. No. 350 (especially pp. 36-37). 1924.

of which the cohesion theory was formulated, Dixon¹ made some observations on daily changes in diameter of woody stems with the pair of calipers shown in figure 1, which are illustrated for the first time. This instrument has been recently loaned the authors by Professor Dixon. In its operation the set screws were placed in bearing on opposite surfaces of the stem. The changes in position of lines made on silvered surfaces on the ends of the arms, denoting expansion or contraction of the stem, were observed by a reading microscope. Although measurements at identical temperatures taken with this instrument, which was of steel with a high coefficient of expansion, would have been comparable, yet Professor Dixon furnishes the information that doubts as to the value of the measurements prevented their detailed publication, as well as of a description of the calipers.

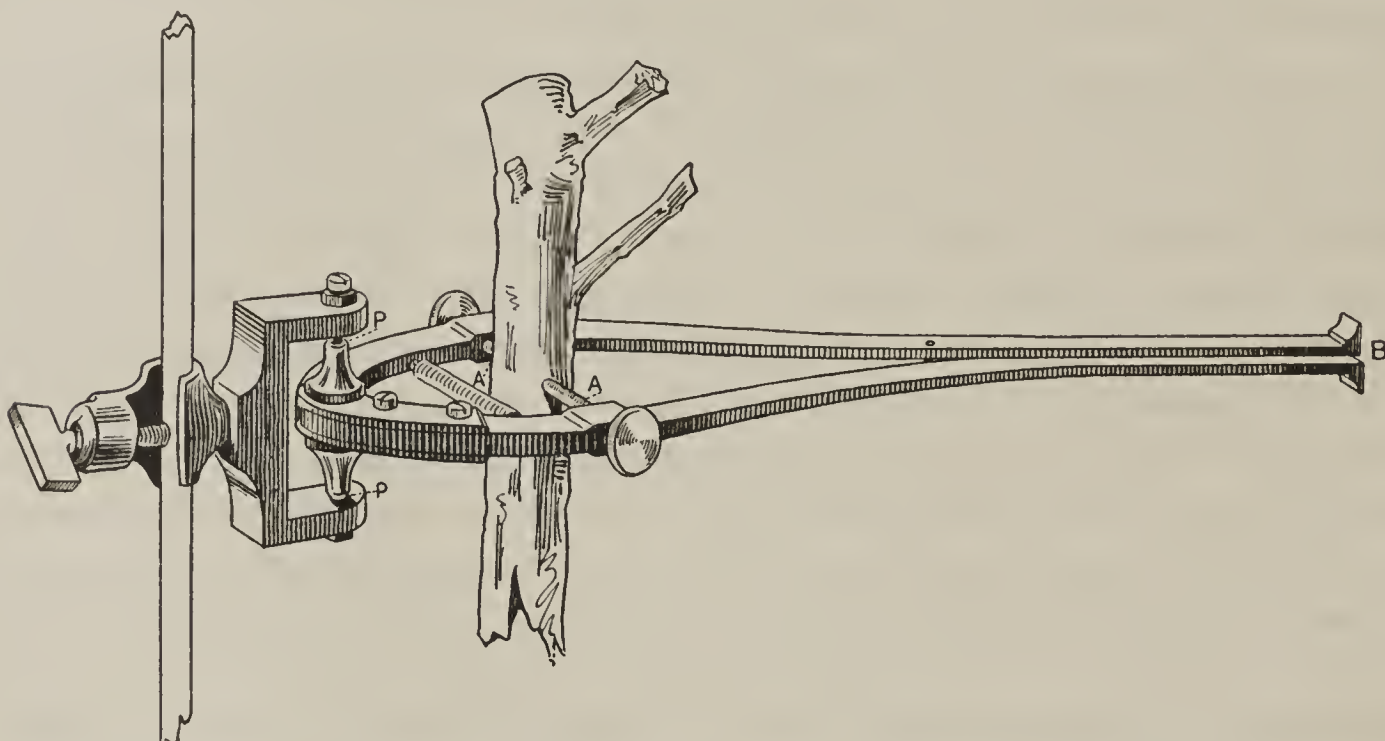


FIG. 1.—Calipers devised by Professor H. H. Dixon, in 1894, to measure changes in the diameter of stems. The screws *AA* are brought into bearing on opposite sides of the stem, and changes in the diameter of the stem are noted by change in position of lines on the outer face (*B*) of the caliper arms, which move on the pivots *PP*. The spring between the screws (*AA*) and the pivots (*BB*) allows the instrument to record both contractions and expansions of the stem.

Friedrich's "Zuwachsautograph" described in 1905² was arranged to record automatically *increases* of stems by small units in a continuous series, but being constructed of metal with a high-temperature coefficient, no data of importance in this connection were secured with it. Modifications of this instrument, have, however, been employed in recent years in Japan.

Mallock,³ using an optical apparatus, observed the changes in dimension of tree-trunks in 1917 by direct observation.

¹ Dixon, H. H., and J. Joly. On the ascent of sap. Phil. Trans. Roy. Soc. London, 186B: 563-576. 1895.

² Friedrich, J. Zuwachsautograph. Centralbl. für das gesammte Forstwesen, 3: 456-461. 1905.

³ Mallock, A. Growth of trees, with a note on interference bands formed by rays at small angles. Proc. Roy. Soc. London, B 90: 186-191. 1919.

The senior author began a series of measurements of the variations in thickness of succulent stems of *Opuntia* in 1915, followed by similar observations on other cacti, on leaves of *Mesembryanthemum*, stems of *Helianthus*, pods of *Phaseolus*, nuts of *Juglans*, and fruits of *Lycopersicum*, in the published accounts of which the shrinkage was attributed to depletion of water by imbibition. The invention of the dendrograph in 1918, and its use in the continuous registration of changes in diameter of tree-trunks of various morphological types, secured records extending not only over the entire day, but throughout the season, and, in some cases, for several successive seasons. Analyses of these records, and of other data, made the connection between the transpiratory draft on the water-mesh in the conducting elements and changes in external dimensions fully apparent. Lessened volume of water under heightened tension was taken to result in decreasing thickness of the conducting tracts.^{1 2 3 4 5}

Meanwhile, Bode⁶ had carried the study of the effect of the varying tension of the cohesive column of water in the conducting tracts to a point where single elements or strands were seen to shrink with increased transpirational pull and to expand when this was released.

With this accumulation of evidence supporting the cohesion theory of sap-flow it became evident that the course of the ascending sap, the identity and location of the elements actually concerned in conduction, and the seasonal adjustments of the transpiration stream, were in great need of detailed study. The minute anatomical details of the conducting elements and the relations of the water-mesh to the gases accumulating in the trunks were in especial need of reëxamination. Much confusion has arisen from attempts to explain the mechanism of movement of sap in one type of stem by results obtained from experiments upon stems of widely different structure and mechanical arrangement of the conducting elements. Trunks of *Salix*, *Quercus*, *Alnus*, and *Juglans* are identical in the fact that water is pulled up by suction set up in the leaves, and that the strands of water are cohesive. As will be shown on a later page, there are marked differences among them as to the portion of the annual layer through which the transpiration stream moves. The transpiration stream may move through late summer wood only, through early spring wood only, or through both early spring and late summer wood.

¹ MacDougal, D. T. Mechanism and conditions of growth. Mem. N. Y. Bot. Garden, 6: 5-26. 1916.

² ———. Hydration and growth. Carnegie Inst. Wash. Pub. No. 297. 1920.

³ ———. Reversible variations in volume, pressure, and movements of sap in trees. Carnegie Inst. Wash. Pub. No. 365. 1925.

⁴ ———. The hydrostatic system of trees. Carnegie Inst. Wash. Pub. No. 373. 1926.

⁵ ———, and Forrest Shreve. Growth in trees and massive organs of plants. Carnegie Inst. Wash. Pub. No. 350. 1924.

⁶ Bode, H. R. Beiträge zur Dynamik der Wasserbewegung in den Gefässpflanzen. Jahrb. Wiss. Bot., 62: 92-127. 1923.

This extreme diversity in the movement of the stream through various parts of the annual layer shows that generalizations as to the path of the stream and its movements are extremely hazardous. The stems under consideration differ markedly in the proportions of conducting and non-conducting elements within the xylem; in length, pitting, and distribution of the vessels and tracheids through which the transpiration stream moves; and in the extent to which conducting elements may be blocked by tyloses. Although there is this diversity of anatomical organization in the xylem, it is not clear how far these anatomical differences affect the distribution of the transpiration stream within the various parts of each annual layer.

The cohesive column of water is subject to continuous variation. First, a rapid rate of transpiration accelerates movement of water upward and increases the tension in the filaments of water; and as a result of an increased pull and withdrawal of water from conducting elements some of the vessels or tracheids, which may be a part of the column at low tension, are emptied and gases are drawn into their cavities. Diminution of tension resulting from decreased water-loss in the leaves might be followed by a reoccupation of some of the gas-filled elements by water with a partial or total solution of the gases and a possible extrusion of any remainder. This action would be a contributing factor to the accumulation of water in trunks at the end of the growing-season when the leaves are shed and transpiration is reduced to a minimum. Whether this cause alone would set up positive pressure is not known; the action in question might precede or coincide with the hydrolyzation of starch in the rays and xylem parenchyma during the spring season, which would bring in additional water with resultant compression by osmotic action. Pressures of 1.0 to 1.1 atmospheres set up in this manner in *Salix* have been recorded in December and January. This feature is also well marked in *Juglans*, in which pressures of 0.6 atmosphere have been recorded in the midwinter resting period at which time the temperature of the air may fall to near the freezing point.

The entrance of water in elements or tracts containing gases under pressure greater than that of the atmosphere would evidently not cause the tract to be included in the cohesive column in active transpiration unless the given vessel or tracheid becomes completely filled with water. As soon as water-loss begins to set up a pull of any magnitude, water would be withdrawn from the elements in which it had compressed the gases, and positive pressures would lessen. In fact, as will be described on a later page, it is the presence of air in some of the vessels and tracheids which delimits the ascending current.

The accumulation of gases in any tracheid or vessel may be taken to cause its elimination from the cohesive mesh as soon as suction

from the leaves is sufficient to pull the air through the minute perforations connecting with other elements above or below. Much attention has been given to the explanation of ascending sap in vessels containing some air with the water in the form of the "Jaminian chain," but it is now known that such a system may not serve for the ascent of water resulting from water-loss which would set up tensions of any magnitude. When the trunk is filled with water in the autumn after the leaves are cast off it is highly probable that the expansion of the water-column or meshwork does not result in filling the vessels and tracheids completely; the conditions of the "Jaminian chain" might now prevail, and slow withdrawal of water by suction of low intensity might take place. Formation and expansion of leaf-surfaces with resultant sudden increase of transpiration would result in the withdrawal of water from some of the tracts filled with water during the season of lesser activity. It is quite possible that air-bubbles in such vessels partially filled with water may have led some observers to assert the presence of bubbles in the ascending column. In some cases these assertions have followed the use of faulty technique.

Bode¹ made microscopic examination of the vessels of wilted plants such as *Impatiens sultani*, *Tradescantia zebrina*, *Elastostemma sessile*, *Cucurbita Pepo*, *Syringa vulgaris*, *Lycium halimifolium*, *Taxus baccata*, *Ginkgo biloba*, *Phaseolus*, *Sambucus* and *Helianthus*, and found that the water-columns remained unbroken in wilted plants, and that gas-bubbles entered the conducting elements only when living cells in contact with them were touched or when other wounding operations were performed. When wounding was performed under mercury, no air-bubbles entered the elements carrying the cohesive column, and no case of separation of bubbles from gases in solution was seen. Holle² had reported earlier that even in leaves completely wilted intact water-columns were to be seen in the conducting elements.

Bode would conclude that bubbles may appear in a vessel containing water only by being drawn in or by wounding. Benecke and Jost³ hold that when suction sets up subatmospheric pressures in water-filled elements some of the dissolved gas may be freed and take the form of bubbles, a view with which the conclusions set forth in this paper are in agreement. It is not clear what is meant by their statement that if the rarefaction continues air will be pulled in through the walls of the vessels. If it is implied that gases may be pulled in through the perforations connecting the vessels in series, then the facts presented in this contribution would be in concordance. Finally,

¹ Bode, H. R. Beiträge zur Dynamik der Wasserbewegung in den Gefäßpflanzen. Jahrb. Wiss. Bot., 62: 92-127. 1923.

² Holle, H. Untersuchungen über Welken, Vertrocknen und Wiederstraffwerden. Flora, 108: 73-126. 1915.

³ Benecke, W., and L. Jost. Pflanzenphysiologie. Vierte Aufl. Jena. 1924.

the statement of these authors that water is found in all vessels is untenable. Numerous observations of ours show that no liquid whatever may be present in certain vessels of *Salix*, *Juglans*, or of *Alnus*.

Some exception must also be taken to the statement of Benecke and Jost to the effect that gases in vessels come into the plant dissolved in water. Some oxygen and more carbon dioxide probably do enter the plant in this manner. The numerous analyses of gases drawn from trunks of *Pinus*, *Quercus*, *Salix*, *Juglans*, and *Populus* from 1925 to 1928 show that the total amounts of oxygen and carbon dioxide present in such gases rarely make more than 22 per cent of the volume of the extracted gas, and that the carbon dioxide content is least in the inactive season and greatest in the period of most active growth. It has also been found that the proportion of carbon dioxide in the included gases increases during periods in which the temperature would be favorable to respiration, while rains or high relative humidity would lessen transpiration, and the water-column would become more nearly saturated with gases. All of these circumstances suggest that a large proportion of the carbon dioxide in the vessels and tracheids originated by respiration of the ray-cells and wood parenchyma, and by possible maturation changes in cellulose walls. The high solubility of this gas would cause much of it to be taken up by the sap and carried to the leaves. The photosynthetic importance of gases carried to the leaves in this manner is, however, yet to be estimated. Rapid transpiration would draw a swift sap-stream past the respiring cells, with a consequent reduction of the carbon dioxide which might be extracted from a bore driven in the trunk.¹

The entrance of water into elements not occupied by the cohesive meshwork in which upward movement takes place in the season of growth and greatest activity is most noticeable in trees which shed their leaves in the autumn, and in which the rate of water-loss would be reduced to a fraction of the maximum. The resultant compression of the undissolved gases was noted in *Salix* and in *Juglans* at the Coastal Laboratory. These trees cast their leaves in November and December. The common oak of this region (*Quercus agrifolia*) is "evergreen," and each suite of leaves is retained throughout the cooler resting period and until the suite of the next season unfolds. Transpiration being carried on at a rate modified by temperature and relative humidity, the increase of water in the trunk of this tree has not been found sufficient to set up positive pressures as in the trunks of the willow and the walnut.

Many conifers, including the Monterey pine and the redwood,

¹ See also in this connection:

Noll, F. Ueber die Luftverdünnung in den Wasserleitungsbahnen der höheren Pflanzen. Sitzungsber. Niederrhein. Gesell., 1897: 148-153. 1897.

Devaux. Sur une action permanente qui tend a provoquer une tension négative dans les vaisseaux du bois. Compt. Rend. Acad. Sci. Paris, 134: 1366-1369. 1902.

which we have studied especially, present cases of continuous transpiratory activity. The xylem of the redwood (*Sequoia sempervirens*) contains so much water that the extraction of gas samples is practically impossible. The comparatively great size of the perforations in the bordered pits would, apparently, allow gases to pass readily from one tracheid to another. The trunks of this tree present the anomalous condition of yielding gases so readily to water-filled bores to which manometers are attached that no manometric measurements can be made for more than a few hours without readjustment, while at the same time the extraction of gases from such bores by direct suction of 0.6 to 0.8 atmosphere, by the method described elsewhere,¹ is impracticable in quantities sufficient for analysis.

On the other hand, the trunk of the Monterey pine, whose xylem is likewise made up largely of tracheids, contains gases in quantities sufficient to be extracted by suction of 0.6 to 0.8 atmosphere. The rate at which gas can be extracted from trunks of this species, which retains its transpiratory mechanism throughout the year, remains approximately the same throughout the various seasons. The rate of the ascending column of water in the wood of the last three or four years, with which the leaves are connected, is much more rapid than in the layers interior to these. No noticeable increase of water has been found in trunks of this species at the end of the growing-season.

It is plainly evident that many generalizations as to the path and movements of sap must be held in abeyance until a more inclusive survey can be made of organization and of the actual path of liquids moving upward in the trunks of trees. This statement is based upon the diverse conditions uncovered in our detailed study of the behavior of trunks of *Salix*, *Quercus*, *Alnus*, *Juglans*, *Pinus*, and other genera as described in the following pages.

¹ MacDougal, D. T. The hydrostatic system of trees. Carnegie Inst. Wash. Pub. No. 373, p. 50. 1926.

THE ORGANIZATION OF A WOODY STEM

Before turning to a detailed discussion of the path and of the forces involved in the movement of the transpiration stream, it may be well to consider the structure of the woody stems upon which our experiments have been performed, and to recall the means by which these stems increase in length and in thickness. The methods by which successive annual increments are added to the xylem is essentially the same in gymnosperms and in perennial dicotyledons, and the subtended generalized account is applicable, with slight modifications to the development of the stem of *Salix*, *Juglans*, or of *Pinus*.

The extreme tip of a woody stem is composed of isodiametric embryonic cells, many of which are dividing. Cross-sections at this level (the apical meristem) show that all of these cells are essentially alike, and that there is little if any differentiation of the embryonic apex into cortex, vascular cylinder, and pith.

When stems are viewed in longitudinal section (fig. 2) the first discernible differentiation of parts is the appearance of mamillate protuberances, the primordia of leaves, a short distance back from the apex. The formation of the leaf-primordia marks the differentiation of the stem into nodes and internodes, a differentiation that is at first scarcely noticeable, since the nodes are close together, but one which becomes more and more marked as the region of elongation posterior to the apical meristem increases in length. The vacuolization and increase in size of these young internodal cells, the sum total of which results in elongation of the internode, is not the same across the internode. At the center of the elongating internode the embryonic cells increase in size equally in all three dimensions, and the pith maturing from these cells is, consequently, composed of isodiametric, approximately cubical cells. Similarly, also, cells at the periphery of the internode enlarge equally in all dimensions and mature into a cortex of approximately isodiametric cells. Cells in the zone between immature pith and cortex enlarge much more in their longitudinal than in their radial or tangential axes, and so constitute a sharply defined though embryonic tissue, the *procambium*, that completely encircles the stem a short distance in from the surface of the internode. The procambium is the embryonic vascular cylinder, and not, as might be inferred from its name, the forerunner of the cambium.

Coincidental with, or shortly after, differentiation of the procambium in the internode there is an appearance of a procambium in the region of the node. The nodal cylinder of procambium is continuous with, and like that of, the internodal procambium, except for a cordate area (the leaf-gap) inward from each primordium where the embryonic cells are isodiametric instead of vertically elongate. At

the base of the gap there is a strand of procambial tissue (the embryonic leaf-trace), that runs diagonally through the cortex from the procambium to the base of the young leaf, and there connects with a

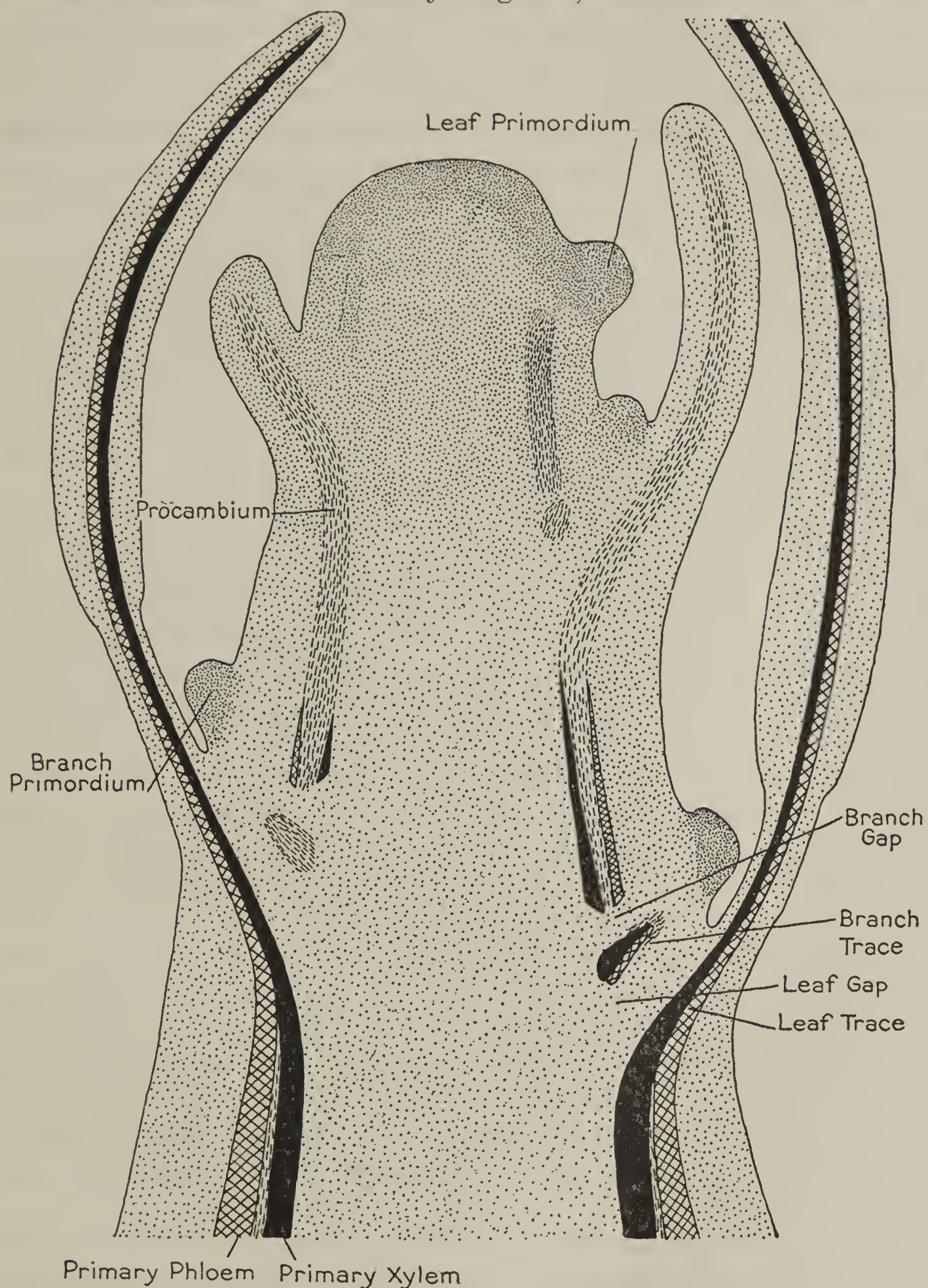


FIG. 2—Diagrammatic longitudinal section through the apex of a woody stem.

similar strand of procambial tissue, the embryonic vascular bundle of the petiole. In *Salix* and *Juglans*, genera that have been especially studied in this investigation, the procambium has three gaps opposite each young leaf, and a single leaf-trace running from the bottom of each gap to the base of the petiole.

Primordia of lateral branches formed by the stem are first evident as hemispherical humps of meristematic tissue, axillary to young leaves, shortly before or just after the stem's procambial system becomes differentiated. The subsequent development of the branch primordia is much slower than that of the stem's apex, and during the season in which they are formed they rarely develop beyond the stage where they bear primordia of foliage leaves and an enclosing sheath of scale leaves. These lateral buds may develop into branches the next growing-season, they may remain dormant for several seasons, or they may never develop into branches.

As mentioned above, the procambial cylinder is interrupted by a gap, or by gaps, in the portion of the node interior to the leaf. If the leaf develops an axillary branch primordium the nodal organization is even more complicated since there is a branch-gap in the portion of the procambium above the leaf-gap, and at the base of the branch-gap two procambial strands (immature branch-traces) that connect the branch primordium with the procambial cylinder.

Following the differentiation of the procambial system in the stem there is a maturation of the procambial cells into a vascular tissue which, since it is formed by the direct maturation of procambial cells, is known as the primary vascular tissue. The first procambial elements to mature into xylem are those immediately adjacent to the pith. As the stem becomes older there is a maturation of abutting procambial elements into primary xylem, and the process is continued until the inner half of the procambium has matured into primary xylem. Coincident with the formation of the first primary xylem elements there is a maturation of the outermost procambial cells into primary phloem and, following this, an inwardly progressive maturation of other procambial elements into phloem until the primary phloem and xylem are separated by a narrow zone of embryonic cells, the cambium. Meanwhile, there has been a maturation, in the same order of sequence, of procambial cells of leaf-traces and of leaves. This maturation into primary tissues, unlike that in the procambial cylinder, continues until primary xylem and primary phloem abut on each other. The vascular bundles of leaf and leaf-trace are, therefore, without a cambium, and consequently are incapable of further increase in diameter.

The shape of the primary vascular system, except for a much greater length of the internodes, is identical with that of the procambial system from which it is matured. It is, therefore, a hollow cylinder that is continuous through the internodes. At the nodes it is interrupted by parenchymatous areas (leaf-gaps) that lie immediately above the vascular bundles (leaf-traces) connecting the vascular cylinder with the bases of leaves (fig. 3).

At the beginning of the changes described above the stem's apex is

hemispherical in shape and composed entirely of embryonic cells. Differentiation and maturation of cells at the posterior portion of the original hemisphere leads to a greatly elongate cylinder of maturing or mature primary tissues. Practically all of the stem's growth in length, externally evident by an elongation of its upper internodes, takes place during this maturation of the primary tissues; after the primary tissues are fully matured there is but slight elongation.

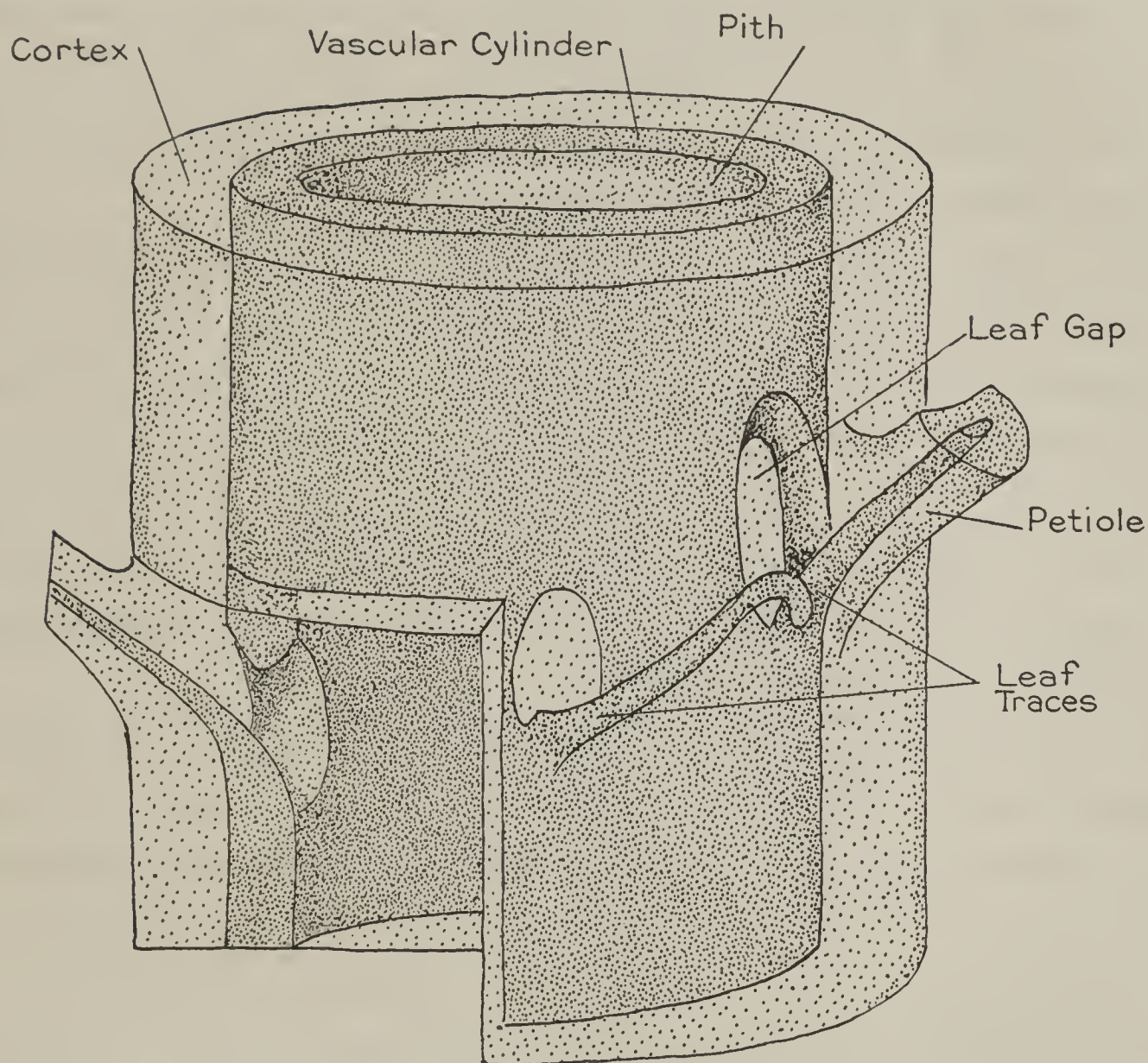


FIG. 3—Nodal organization of the primary tissues of *Salix* (diagrammatic). The cortical tissues are represented as semi-transparent to show the darker-shaded vascular tissues internal to and passing through the cortex. The lower left-hand portion of the stem has been cut away to show a portion of the stem in longitudinal section.

Completion of the direct maturation of embryonic cells into primary tissues does not mean a cessation of formation of new elements at any node or internode since the cambial cells begin to form secondary xylem and secondary phloem shortly after the primary vascular tissues are mature. The first secondary xylem elements are laid down just outside the cylinder of primary xylem, later-formed secondary xylem elements are laid down external to the already formed secondary xylem. Continued formation of secondary xylem results in a constantly thickening cylinder of secondary xylem, and one in which the oldest elements are at the inner face and the youngest elements next the outer face. The first-formed elements of the sec-

ondary phloem lie just inside the cylinder of primary phloem, and later-formed secondary phloem elements are internal to the earlier-formed ones. Thus the secondary phloem shows a reverse condition from the secondary xylem, and the older elements are at the outer face of the cylinder of secondary phloem and the youngest elements at its inner face. Cambial activity of the internodes results only in a gradual thickening of the vascular skeleton. At the nodes, however, there is a gradual extension of cambial activity across the face of the gaps, and, with the continued formation of secondary tissues, a gradual closing of the gap. Thus, at the end of the first growing-season the outer face of the xylem is a continuous cylinder, and not a perforate sheet as when first differentiated.

The primary tissues are differentiated early in the growing-season, and secondary thickening continues throughout the season. As a result the volume of secondary xylem is much greater than that of the primary xylem, and the secondary xylem is, therefore, of much greater importance as a path for the transpiration stream than is the primary xylem. The sheath of secondary xylem is not of uniform thickness throughout its length, but is much thicker at its base than at its apex. Thus, at the end of the first growing-season the stem is an elongate cone, with gradually tapering sides, and a rounded apex, the undifferentiated apical meristem.

The next growing-season the cambium renews its activity, and secondary xylem and phloem are laid down in the same succession as in the first season. The cambium gives rise to the same types of secondary elements as in the first season, but there are marked differences in size and arrangement between those formed in the latter part of the first season (the summer wood) and the first-formed elements of the succeeding season (the spring wood). There is, consequently, a sharp line of demarcation between the secondary xylem of the two seasons.

At the beginning of the second season the leaf-traces of the previous season are intact, despite the fact that the leaves to which they lead are no longer present. Soon after the cambial activity is renewed the radial tensions on phloem and cortex, occasioned by the formation of additional secondary xylem, rupture the trace where it traverses the cambium. The exposed end of the trace is then overlaid and buried by the secondary xylem of the second season. The severed portion may remain intact in the cortex for several seasons, but it becomes more and more remote from the secondary xylem with the continued formation of phloem by the cambium. When these old traces are considered as possible paths for lateral transfer of the transpiration stream, there is the possibility of a movement of water from the first to the second year's wood unless there are structural changes in the old trace preventing the movement of water.

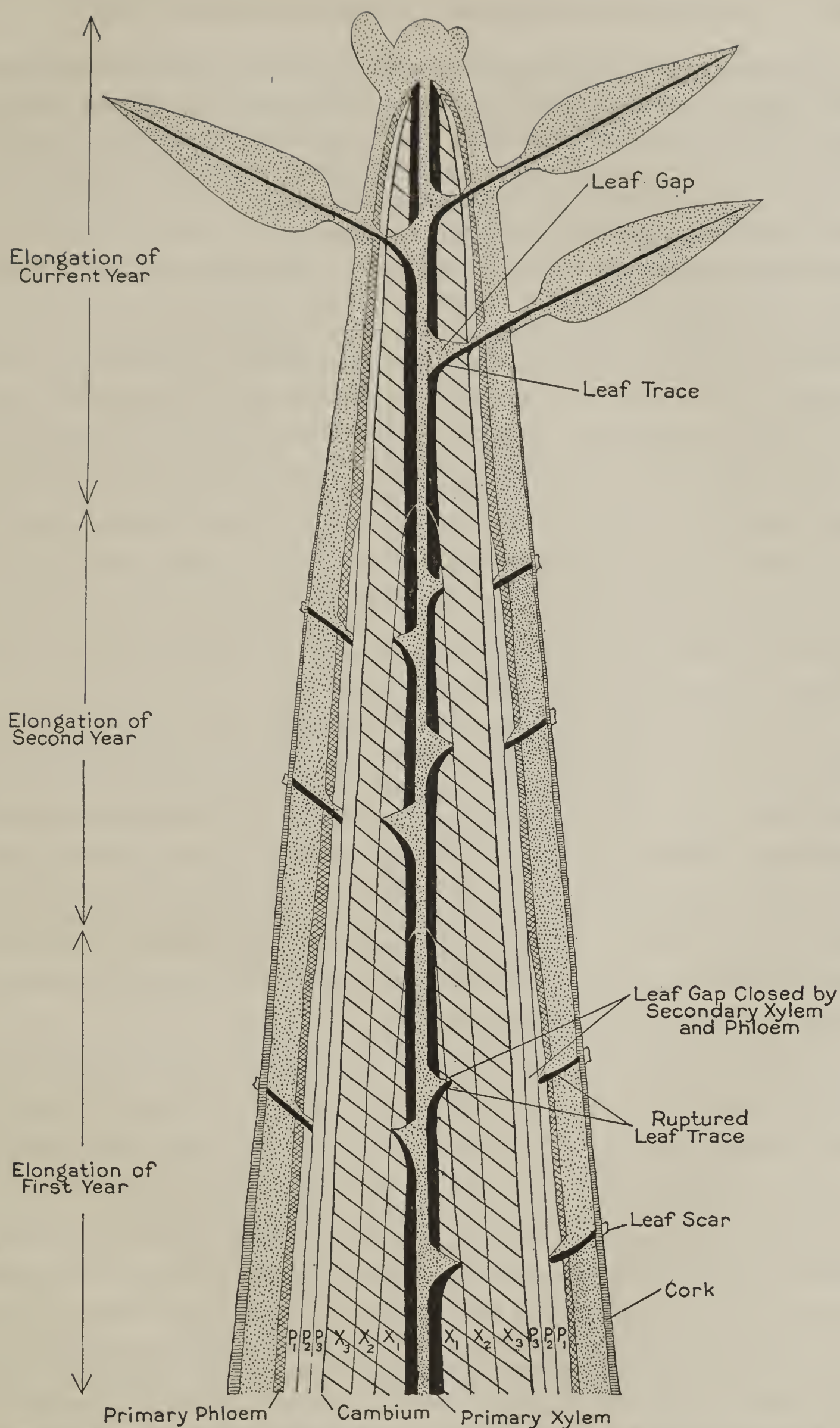


FIG. 4—Diagram of a longitudinal section of a three-year-old woody stem. X_1 , X_2 , X_3 , and P_1 , P_2 , P_3 are, respectively, the secondary xylem and secondary phloem of the first, second, and third years. Branch primordia and branches have been omitted from the drawing to avoid undue complexity.

Coincident with this lateral growth external to the previous year's wood there is an elongation posterior to the apical meristem, and the same differentiation of primary and secondary tissues as in the first year. The cylinder of primary xylem differentiated the second season is of the same diameter as that formed during the first, and, from the structural standpoint, constitutes a vertical extension of the first year's primary xylem cylinder. (Fig. 4.) Secondary xylem formed in the region of longitudinal extension of the current year is not continuous with the secondary xylem of the previous year, but with secondary xylem external to it. Thus, the tissues of the second year's growth, when taken by themselves, constitute a cone whose upper half (the longitudinal growth of the second season) is solid, and whose lower half (the portion lateral to last year's wood) is hollow.

New tissues formed during the third and succeeding years repeat the history of the second year. The previous year's wood is ensheathed by an additional layer of secondary tissue, and there is a vertical elongation of the stem's apex with a subsequent formation of primary and secondary tissues. Thus, except for greater length below the solid portion, the tissues of subsequent years have the same conical form as those of the second year.

The above-described longitudinal extension and lateral thickening of the stem is usually accompanied by a development of lateral branches. As already noted (page 16), the primordia of lateral branches arise axillary to leaves and at the time the stem's procambial system is differentiating. The branch primordia are connected with the stem's procambial system by branch primordia, two to each primordium, and there is a branch-gap above the point of departure of each pair of traces. Branch primordia are identical in organization with apical meristems, and differ only in the time at which their cells begin maturing into primary tissues.

Under exceptional conditions, as in young shoots from dormant buds on old willow stems, a branch primordium may develop into a lateral branch the same year that it is formed. If there is this precocious development of a primordium its primary tissues abut directly on the primary vascular tissues or upon the early-formed secondary xylem and phloem of the main stem. Ordinarily, however, all primordia remain dormant during the first season, and the secondary wood of the first season's growth buries the branch-traces in the same manner that it buries the leaf-traces. On the other hand, there is not a closing of the branch-gap since the gap develops a cambium-like layer that adds new parenchymatous cells as the secondary xylem increases in diameter. If the bud does not develop into a branch the next season the lateral extension of the gap continues, and it is not at all uncommon to find, as in the willow, branch-gaps that run horizontally through a dozen or more annual rings.

Although each leaf has an axillary branch-primordium, certain ones only of these develop into lateral branches the next season. The factors causing certain primordia to develop into branches and others to remain dormant are not understood. They are not the same in all cases. Thus, in the walnut the primordia that develop into branches are usually those near the apex of last year's growth. In the willow, on the other hand, basal primordia are as apt to develop into branches as those near the apex.

A two-year-old stem usually forms several lateral branches. The following season, when the branch-bearing portion is three years old, all of these branches may grow and form a new set of leaves. Or, as is more commonly the case, certain of these branches may grow and others die. In certain one-year-old branches of *Salix* and *Juglans* this failure to develop is due to the formation of an abscission layer that soon causes an amputation of the young branch. In other branches the reason for the failure to develop is not apparent, and the dead one-year-old branch may persist for several years.

The one-year-old branches that remain alive have the same ontogeny as one-year-old stems. That is, there is a vertical elongation, a formation of new leaves at the apex of the branch, a formation of an additional layer of secondary tissue lateral to the first year's growth, and a development of certain lateral buds into branches. The further growth of the stem and its branches is but a repetition of the process described above. Every year there is an elongation at the apex of the stem and of each branch and the addition of a new cylinder of secondary tissue lateral to the old parts of the stem and the branches. The cylinders of secondary xylem developed by stem and branch during any given year are continuous with one another, and they remain continuous after they have been overlaid by successive yearly increments of secondary xylem. Thus the transpirational pull through any given annual ring of a branch draws only upon the corresponding annual ring of the stem. This is true even for the oldest yearly cylinders of branch and stem; cylinders whose juncture has been deeply buried in the stem by many later-formed cylinders of secondary xylem.

Since our experiments confirm the widely held view that the movement of the transpiration stream is exclusively through the xylem, and since these experiments also show that the path of the stream through the xylem is not the same for the various plants that we have studied, a description of the organization of the xylem of the trees used in our experiments is essential to a thorough understanding of the movement of the transpiration stream. This description necessarily centers around the origin and organization of the secondary xylem, for, in the case of stems more than a year old, the primary xylem is an insignificant factor in a consideration of the pathway of the transpiration stream.

The secondary xylem is a tissue resulting from the maturation of derivatives cut off towards the inner face of the cambium. Radial or front face views of the cambium, demonstrated by tangential sections of the stem, show that it is in part composed of isodiametric cells (the ray-initials) and in part composed of vertically elongate embryonic cells (the fusiform initials).

In the willow the vertically elongate fusiform initials lie lateral to one another with their long axes paralleling the surface of the stem. These initials are not in regular rows transversely around the cambial cylinder, but lie so that the ends of one initial are in lateral contact with the middle portion of abutting initials.

The ray-initials of the willow are in vertical files, one cell broad, in which the terminal cells of the file are triangular in face view and the median cells rectangular. The various files of ray-initials are laterally separated from one another by three to eight intervening fusiform initials. When first formed the young derivatives are thin-walled and with dense protoplasts. All derivatives, excepting the uppermost and lowermost in the file, then become vacuolate and enlarge in the axis radial to the stem's center. These cells when full-grown are usually two to three times as long as they are broad, and with the long axis radial to the center of the stem. The uppermost and lowermost derivatives in the file enlarge both radially and vertically. In these marginal cells the vertical axis is the longest. Following the enlargement of the derivatives from the ray-initials there is a deposition of secondary wall-layers. This thickening is not uniform on walls abutting on other ray-cells, and there are, here and there, small circular areas (simple pits) where there is no secondary thickening of the wall. The protoplasts of ray-cells do not disappear after maturation is completed, but generally remain alive for many years after the cells are matured.

Derivatives from the fusiform elements in the cambium of the willow mature into vessels, wood fibers, or into wood parenchyma. A vessel results from the maturation of a vertical series of cambial derivatives that stand one above the other. Shortly after these derivatives are formed by the cambium there is a lateral enlargement of each cell in the vertical file to several times its original diameter, but there is no corresponding increase in length. At the end of the period of enlargement these cells are still separated from one another by diagonal end-walls. Soon after the enlargement is completed large circular perforations, approximately the same diameter as the cells, appear in the diagonal end-walls. Thus, the vertical file of maturing derivatives becomes a single physiological unit, the vessel; a unit in which each cambial derivative is still discernible and known as a vessel segment. The secondary wall-layer deposited on the lateral walls of the vessel segments during the later stages of maturation may

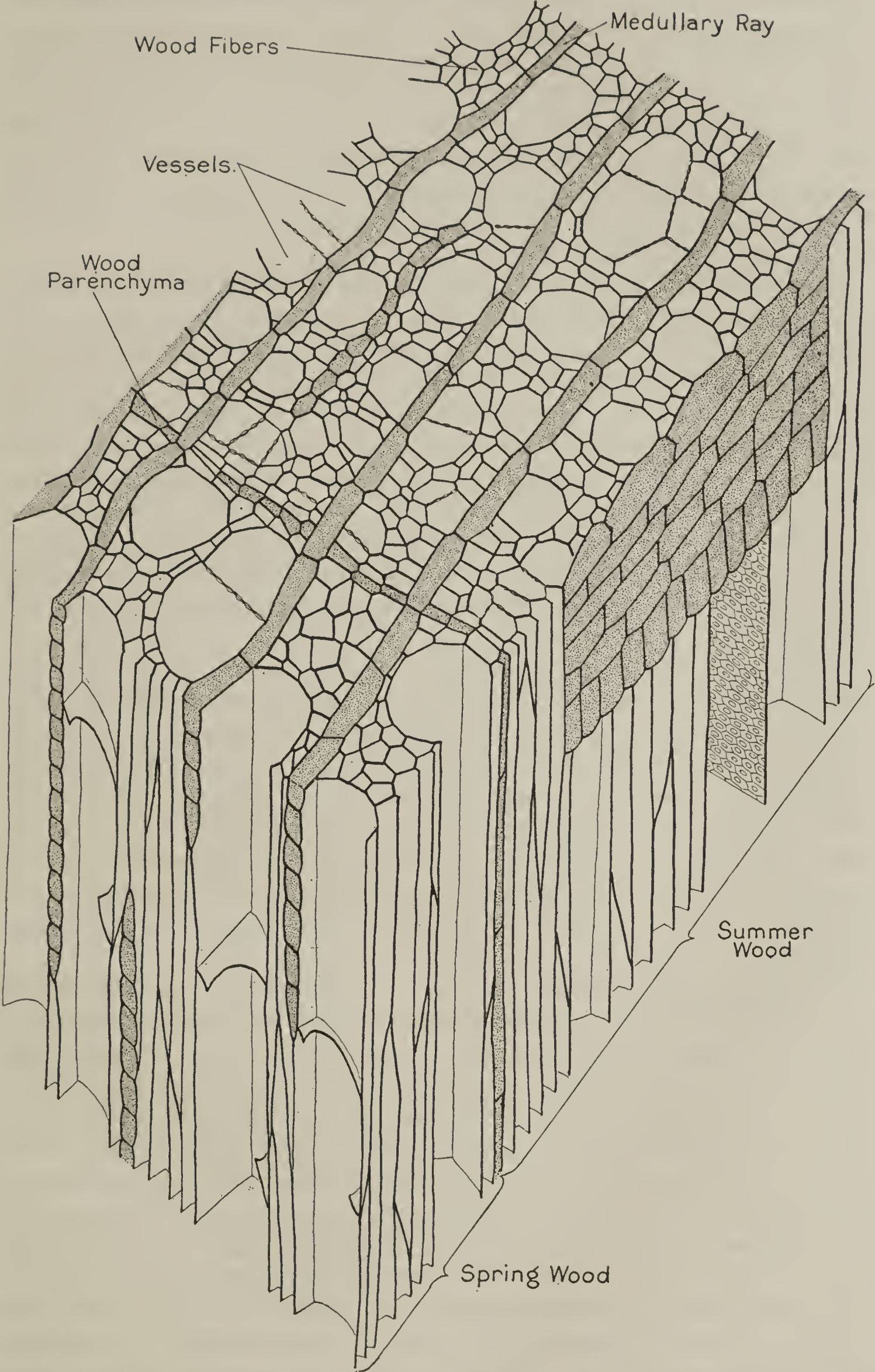


FIG. 5—Portion of the secondary xylem of *Salix lasiolepis*, viewed in three dimensions

be smooth or pitted, depending on the secondary xylem with which the segment is in lateral contact. Faces of segments lying next to wood fibers do not develop pits. If the segment is in lateral contact with a segment of another vessel there is a development of bordered pits on the abutting walls; portions of vessel-segment walls in contact with xylem ray-cells develop half-bordered pits. After the secondary thickening of the walls is completed the protoplast disappears from each vessel segment. Mature vessels are, therefore, devoid of living contents. (Fig. 5.)

Two or three tangentially adjoining files of derivatives may mature into vessels at the same time. When this occurs the vessels in the mature wood abut tangentially on one another. More commonly two or three successive derivatives from a vertical file of cambial cells mature into vessels. This results in a row of vessels radial to the stem's center. The willow has a fairly uniform distribution of vessels from the spring wood formed early in the growing-season to the summer wood formed late in the growing-season. Such a diffuse-porous wood does have, however, great differences in the size of vessels in spring and summer wood of any annual increment, and vessels of the early spring wood are two to three times the diameter of those in late summer wood.

Each wood fiber in the secondary xylem of *Salix* is the product of a single cambial derivative. When a derivative starts maturing into a wood fiber there is, first of all, a longitudinal elongation until the young cell grows to double its original length. Derivatives maturing during the spring also enlarge somewhat in the axis radial to the stem's center, but those formed later in the growing-season do not have this radial enlargement. Following the enlargement of the young fiber there is the formation of a thick layer of secondary wall material, after which, in the majority of cases, the protoplast disappears.

Daughter-cells from the division of the fusiform cambial initials at the close of the growing-season may mature into wood parenchyma instead of maturing into vessels or wood fibers. Shortly after such derivatives are cut off by the cambium they divide transversely, and further transverse divisions eventually produce a vertical file of rectangular embryonic cells. Each of these cells is thin-walled when first formed; later there is a secondary thickening of the walls that is accompanied by a formation of simple pits of faces abutting on wood parenchyma or xylem ray-cells, and a development of half-bordered pits on faces next to vessels. Wood-parenchyma cells, like the parenchymatous xylem ray-cells, retain their protoplasts for a long time.

The cambium of *Juglans*, like that of *Salix*, consists of fusiform initials and ray-initials. However, since the vertical files of ray-initials are, in many cases, more than one cell in breadth, the xylem

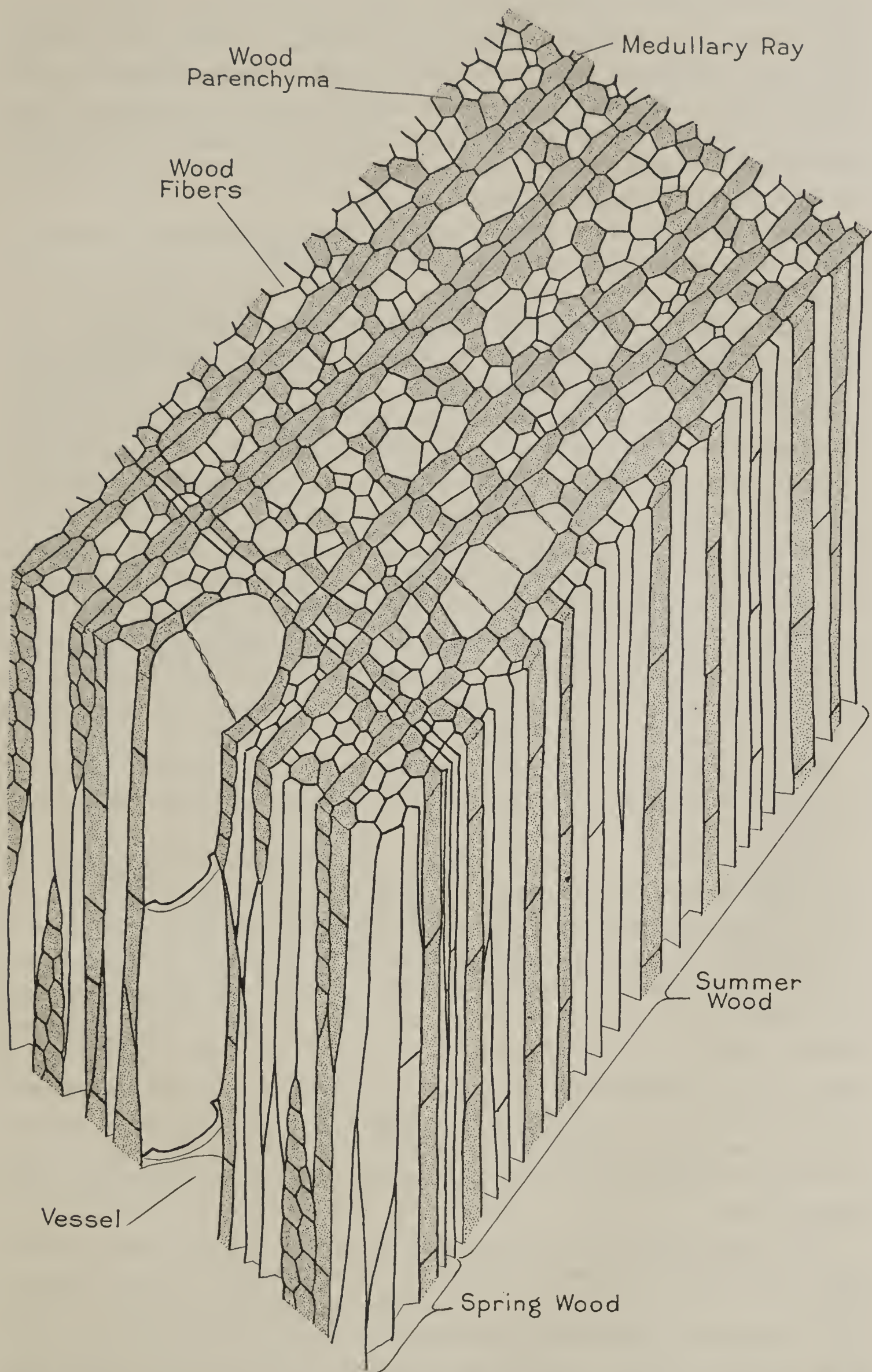


FIG. 6—Portion of the secondary xylem of *Juglans californica*, viewed in three dimensions.

rays of the walnut are broader than those of the willow. The organization of mature portions of the ray is similar to that of the willow, with erect marginal cells and interior cells radially elongate to the stem's center. The structure of the cells in the ray is similar to that in the willow. That is, the marginal cells are elongate in the plane perpendicular to the radius of the stem, and the remaining cells are elongate radially to the center of the stem.

Vessels of the walnut have a length three times that of vessels in the willow, although individual vessel segments in the two genera are approximately the same length. (Fig. 6.) The vessel segments of a walnut have diagonal end-walls, with a single large circular pore, and the same lateral pitting as those of the willow. There are, however, certain differences in size and arrangement of vessels in the two genera. All three species of *Juglans* growing on the grounds of the Coastal Station at Carmel (*J. californica*, *J. major*, and *J. regia*) have more numerous vessels in the spring wood, and these spring-wood vessels may have a diameter ten times that of vessels in the late summer wood. This greater size and number of vessels in spring wood results in a ring-porous instead of a diffuse-porous organization of the secondary xylem. There is also a greater tendency for a vertical file of fusiform cambial initials to produce several successive files of derivatives that mature into vessels. As a result there are frequently radial rows of a half-dozen or more vessels in secondary xylem of the walnut.

The wood fibers of the walnut have somewhat thicker walls than in the willow and a few scattered bordered pits with narrow slot-like mouths.

Juglans, like *Salix*, has a development of wood parenchyma on the outer face of the summer wood of each annual increment. In addition to this formation of terminal parenchyma there may also be a development of fusiform cambial derivatives into wood parenchyma at any time from the beginning to the end of the growing-season. Thus the entire secondary xylem has vertical files of wood parenchyma cells intermingled with the wood fibers; the files of wood parenchyma lie isolated from one another or in transverse plates from one xylem ray to another. There is also more or less formation of wood parenchyma immediately adjacent to the vessels, and, consequently, a complete or incomplete ensheathing of the vessels by wood parenchyma.

The organization of the secondary xylem of the live oak, *Quercus agrifolia*, is quite different from that of the willow or the walnut. (Fig. 7.) First of all, the ray-initials of the cambium form two types of xylem rays: small uniseriate rays one cell broad and three to a dozen cells in height, and large multiseriate rays several cells broad and many cells high. The multiseriate rays are the radial lines divid-

ing the stem into numerous narrow tapering sectors when the stem is viewed in cross-section and without magnification.

The secondary xylem of the oak contains both vessels and tracheids. The individual segments of the vessels are approximately the same size as those of the willow, but the vessels themselves are about four times as long. Vessel segments of the oak have end-walls with the

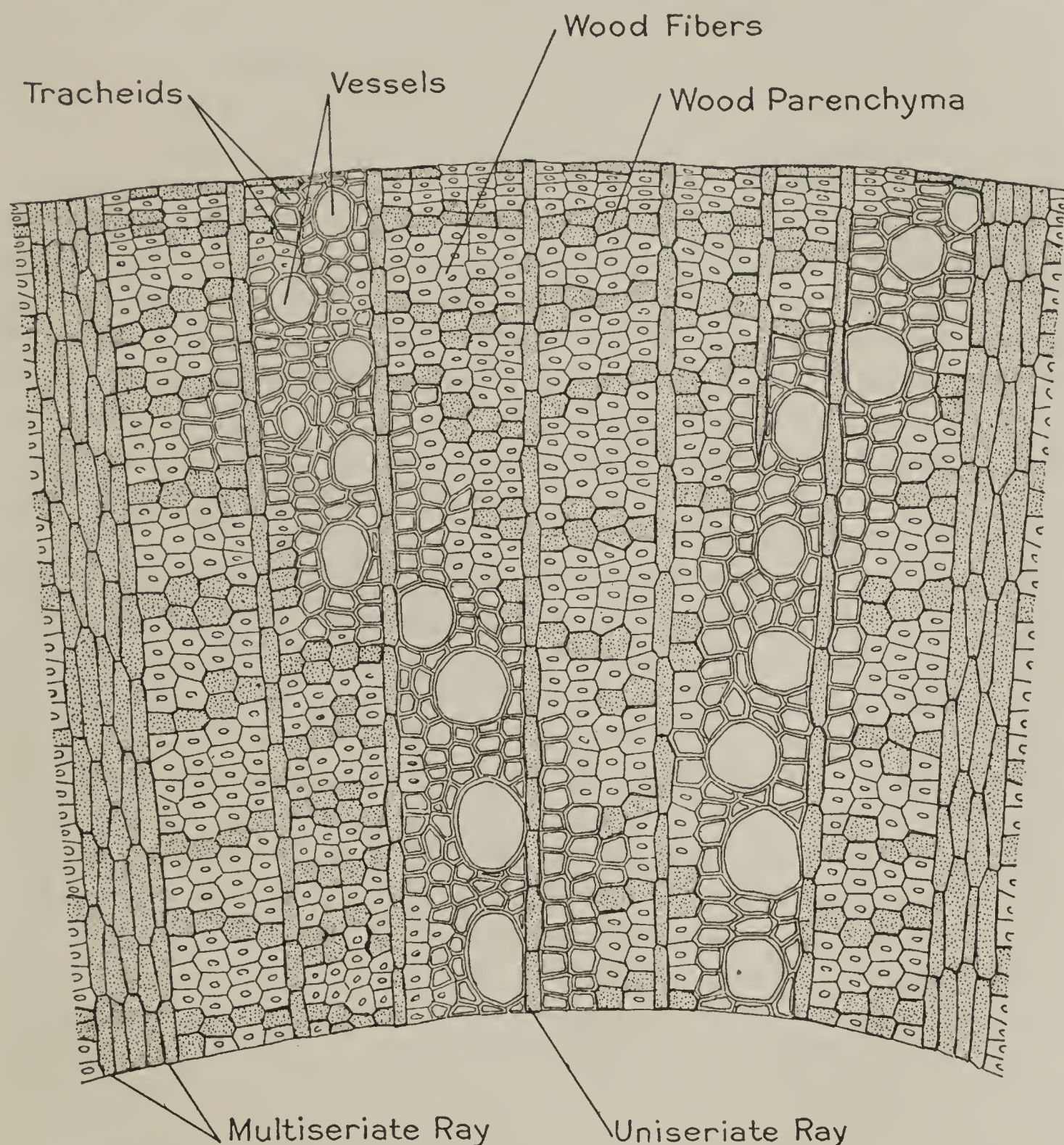


FIG. 7—Transverse section of a portion of an annual layer of the secondary xylem of *Quercus agrifolia* (semidiagrammatic).

same type of large circular opening as in the willow and walnut, but the end-walls themselves are less obliquely disposed with reference to the lateral walls. Most deciduous oaks have much larger vessels in the spring wood than in the summer wood, and, consequently, a sharply defined ring-porous organization. This is not the case with *Quercus agrifolia*, which is “evergreen” (carrying one suite of leaves a full year), and vessels in the spring and summer wood do not differ markedly in size.

Quercus agrifolia also has a more pronounced radial zonation of the vessels than do most other species of the genus. In this oak the portion of an annual layer between two multiseriate rays has one or two straight or curved radial rows of vessels that run from the inner to the outer portion of the layer. The vessels in any row do not abut on one another, but are radially separated by intervening masses of secondary xylem composed chiefly of tracheids. Lateral to the radial rows of vessels and tracheids there are areas containing wood fibers and wood parenchyma only. Thus, in the sector of an annual layer that lies between two multiseriate rays there are radial strips of water-conducting tissue and radial strips of non-conducting tissue.

The tracheids of the oak are matured from cambial derivatives adjoining cambial derivatives maturing into vessel segments. Each tracheid is the product of a single cambial derivative, and in the development into a tracheid the young derivative elongates to twice its length, and then develops a secondary wall-layer with a pitting similar to that found in vessels.

The xylem parenchyma and wood fibers are quite similar in structure to those of the walnut, and have much the same distribution with reference to each other.

There are no essential differences between the organization of the cambium in gymnosperms and in angiosperms; both consisting of fusiform and ray-initials. However, the secondary xylem formed by the cambium of most gymnosperms is very simple in organization compared with that of angiosperms.

Except for the occasional maturation into resin-secreting cells all fusiform initials of the cambium of *Pinus radiata* mature into tracheids. The young derivative cut off by the cambium increases but little in size either in the vertical axis or the axis tangential to the stem's surface. If it is a derivative formed early in the growing-season, it does, however, enlarge in the radial axis. On the other hand, derivatives formed late in the growing-season have but little of this radial enlargement. Thus, there are marked differences in radial diameter of tracheids in early spring and late summer wood. (Fig. 8.)

The secondary wall-layer laid down by the maturing tracheid develops half-bordered pits on areas abutting on parenchymatous cells of the xylem rays, and bordered pits in faces abutting certain other tracheids. If the tracheid is one in the spring or early summer wood there are bordered pits only on the radial faces of the tracheid. If it is one of the last-formed tracheids in the annual layer there are bordered pits on both the radial and the tangential faces.

Most of the files of ray-initials are in uniseriate vertical arrangement and 4 to 5 cells high, a few files are multiseriate in arrangement and 2 to 4 cells broad. Derivatives from the uniseriate files may

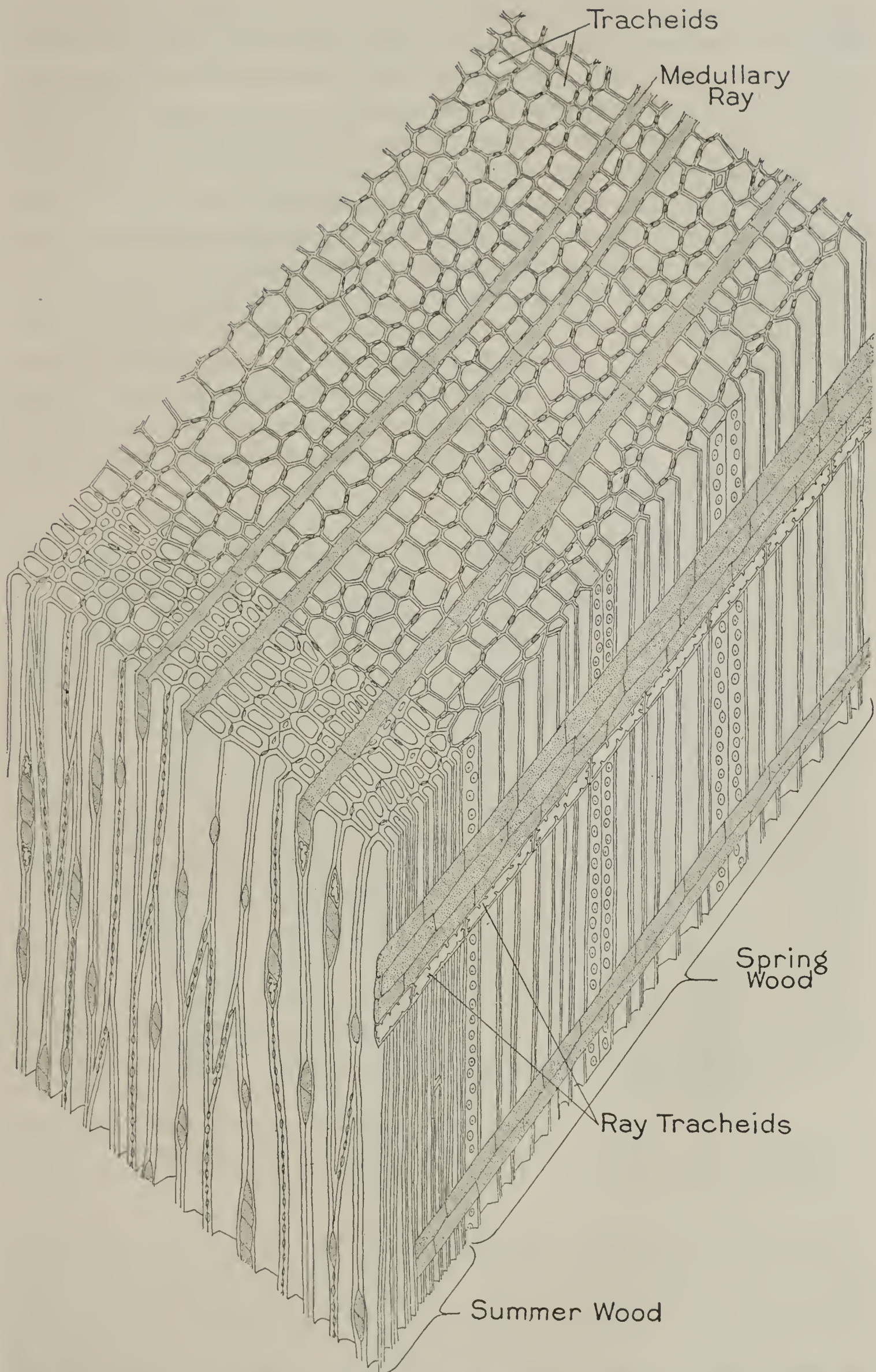


FIG. 8—Portion of the secondary xylem of *Pinus radiata*, viewed in three dimensions.

mature into ray parenchyma-cells or into ray tracheids. Derivatives from the uppermost and lowermost cells of the file generally mature into ray tracheids, and derivatives from the interior cells generally mature into ray parenchyma. Occasionally, however, there is a development of ray tracheids in the interior portion of the ray. The ray parenchyma-cells are similar in structure to those found in angiosperms, and, like ray parenchyma in angiosperms, retain their protoplasts long after they are mature. The ray tracheids have approximately the same shape as the ray parenchyma-cells, but the secondary wall-layers are much thicker, and have small dentate humps projecting towards the center of each tracheid. There is also a development of pits on the various faces of the ray tracheids; bordered pits developing on faces next tracheids or other ray tracheids. Xylem rays formed by a multiseriate group of initials have, in addition to ray parenchyma and ray tracheids, a resin-canal that runs longitudinally through the ray.

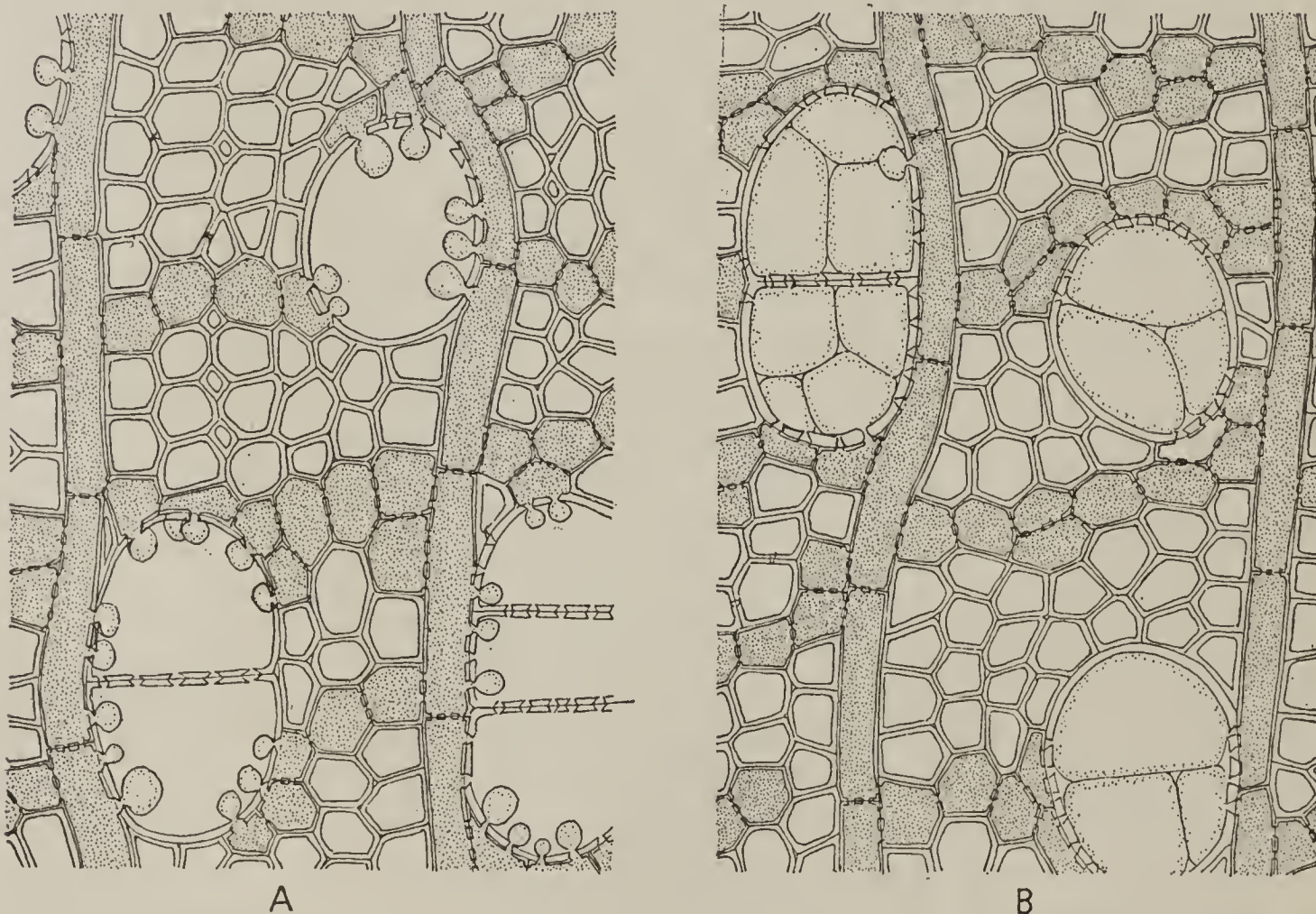


FIG. 9—Transverse sections of portions of the secondary xylem of *Juglans californica* showing tyloses. Figure A shows small tyloses beginning to protrude into the lumina of the vessels; Figure B shows the tyloses completely occluding the lumina of the vessels.

The movement of the transpiration stream through the xylem of a one-year-old stem is not through all elements of the xylem, but only through the tracheids and vessels. A two-year-old stem has the wood of the first year ensheathed by a cylinder of xylem laid down during the second year. This does not mean that vessels and tracheids of the first year's wood are no longer available as a pathway for the

ascending sap, for one finds that the transpiration stream moves through the xylem of both years. Neither does the formation of secondary xylem in the third or succeeding years exclude the possibility of the sap ascending through xylem formed in earlier years. Thus, it is possible to find old stems in which the transpiration stream is moving through all annual increments from the oldest to the youngest.

There may, however, be changes in mature vessels and tracheids that block the movement of water. Sometimes, as in certain tracheids of the pine, the blocking of the transpiration stream is due to a filling of the tracheids with resin. More commonly, especially in vessels of angiosperms, the blocking is due to invaginations from abutting cells.

These invaginations (tyloses) arise only where a parenchymatous cell (either wood parenchyma or ray parenchyma) abuts on a tracheid or a vessel. *Juglans* is particularly favorable for the study of tyloses. Here, as elsewhere, the development of a tylosis begins with the expansion of the closing membrane in a half-bordered pit between the parenchymatous cell and the vessel segment. (Fig. 9A.) The continued expansion of the closing membrane soon results in a small bubble-like outgrowth of the parenchymatous cell, with a lining of cytoplasm, that projects into the lumen of the vessel segment. The young tyloses are spherical in shape. As they increase in size they generally come in contact with one another and become angular by mutual compression. (Fig. 9B.) If the expanding tylosis does not come in contact with other tyloses it remains spherical until it touches the opposite wall of the vessel segment. Thus, by the expansion of a single tylosis or by the mutual contact of tyloses, there is an occlusion of the lumen of the vessel segment that blocks a movement of the transpiration stream through that vessel.

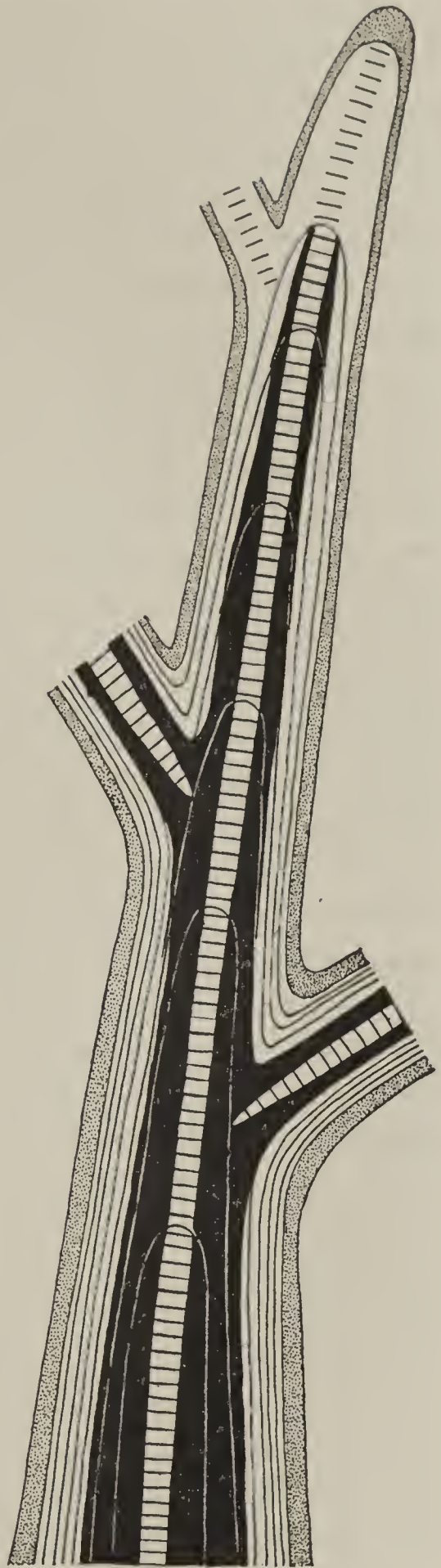


FIG. 10—Diagrammatic longitudinal section of a seven-year-old shoot of *Juglans major* showing (in black) the portions of the secondary xylem that have become tylosed. Untylosed portions of the xylem are in white, and all other tissues are in stipple. Vertical scale 1 to 5; horizontal scale $\times 2$.

Of the genera discussed on the foregoing pages only *Juglans* has a regular tylosing of all vessels in xylem that has attained a certain age. This age is quite different for the various annual increments. The tylosing of the primary xylem, including the leaf-traces buried in the secondary xylem, is completed early in the second year. The occlusion of the remaining portion of the first year's xylem does not usually take place until the stem is three years old. There then follows, usually when the stem is four years old, a tylosing of the second annual ring. This tylosing begins at the inner face of the spring wood, and gradually progresses outward until all vessels in the ring are blocked. Later-formed annual increments attain to a progressively greater age before tylosing. For example, a 15-year-old stem of *Juglans californica* shows rings six or seven years old that are without tyloses. Variations in the amount of tylosing, especially in young stems, is more or less correlated with the volume of the transpiration stream passing through the stem. Thus, three- or four-year-old stems with few lateral branches and a small leaf-surface have much more tylosing than do stems of similar age with numerous branches and leaves. (Fig. 10.)

Salix lasiolepis and *Quercus agrifolia* have but little tylosing of the vessels. In contrast with conditions in *Juglans*, 15-year-old stems of the live oak show no tyloses in the secondary xylem and but few in the primary xylem. There is never a complete tylosing of the first three or four annual layers of *Salix lasiolepis*, only occasional groups of vessels here and there in the layer showing tyloses. The young branches in this willow are subject to attack by a bark-boring insect that penetrates to, but does not destroy, the cambium. This disturbance of the cambium results in a secondary xylem with small circular or linear flecks of brownish callus tissue; flecks that are later buried in the xylem as the cambium exterior to the callus resumes the regular formation of secondary xylem. Since the tylosed areas of the young branch are almost always adjacent to these callus areas, such tylosing as is found in the stem of the willow has been considered a result of the callus formation. Older parts of the stem are not subject to these cambial disturbances, and the older annual rings do not contain the patches of brown callus tissue. Here, consequently, one does not find tyloses.

THE PATH OF THE TRANSPIRATION STREAM: LONGITUDINAL MOVEMENT IN THE STEM

Experiments with dyes and other liquids have been performed to determine the path of conduction in such trees as the oak (*Quercus agrifolia*), the willow (*Salix lasiolepis*), the pine (*Pinus radiata*), certain walnuts (*Juglans major*, *J. californica*, *J. arizonica*, and *J. regia*), and, to some extent, the red alder (*Alnus oregona*). The results of some preliminary studies on the path of transfer of water in stems in the willow and walnut have already been published,¹ together with a description of the methods and experiments used. All experiments show that conduction of dyes, such as acid fuchsin, is very strictly in the xylem and none in the phloem.

Numerous investigators have shown that dyes or other solutions travel in the transpiration stream through the elements of the xylem. When cut branches, stems, or larger portions, such as tree-trunks, are placed in solutions the liquid will ascend to some distance as the plant transpires. The course of the liquid can be easily traced by cutting the stem across at various levels, or by splitting the stem longitudinally.

The specific elements through which the dyes were moving was determined by sectioning the wood and examining the sections under a microscope. Observations made upon sections of freshly cut water-saturated wood, just removed from the dyes, may, perhaps, lead to erroneous conclusions because of the oozing-out and spreading of the dye on the freshly cut surface. This experimental error has been overcome by allowing the treated stems to become thoroughly dry and sectioning without moistening and mounting the sections in paraffin oil, a medium in which the dye is insoluble. Small branches were usually sectioned a month or two after treatment; trunks of trees were allowed to dry a year or more before sectioning. The liquids used by us in tracing the movements include such non-toxic dyes as acid fuchsin, 1 part dye to 1,000 parts of water, and toxic 1 per cent aqueous solution of picric acid. Small branches and stems were severed and allowed to transpire with their bases set in the solutions. On account of the abundance of experimental material present in the garden of the Coastal Laboratory at Carmel, California, not only small stems were used, but the experiments of Strasburger were repeated by sawing off relatively large willow, walnut, and pine trees, and then stepping the severed trunks into the various solutions, allow-

¹ Overton, J. B. Stem anatomy and sap-conduction. Carnegie Inst. Wash. Year Book, No. 25: 155-158. 1925-26.

MacDougal, D. T., and J. B. Overton. Sap-flow and pressure in trees. Science, n. s., 65: 189-190. 1927.

Overton, J. B., and G. M. Smith. Additional observations on stem anatomy and sap-conduction. Carnegie Inst. Wash. Year Book No. 26: 159-160. 1926-27.

ing them to stand in the solution for several days, after which the distribution of the liquid was determined.

We have also studied the distribution of the transpiration stream by substituting the pull of about 1 atmosphere of a suction-pump for that of transpiration. The apex of a stem was attached to a suction-pump, the base of this stem being placed in the solution. In every instance the distribution of the solution thus drawn into the xylem, was identical with that obtained by means of a transpirational pull. The advantage of this method is that one can obtain in a few minutes results which would require a day or more if obtained by means of transpiration.

Objections have been raised against the use of amputated stems for the demonstration of the path of the transpiration stream. Opponents of this method hold that the living plant is a closed system, and that the results obtained by means of cut stems and branches are invalid since the investigator is working with conditions wholly different from those that obtain in an intact plant. We believe that the objections to this use of amputated stems have been overcome by our injection experiments, where, in effect, dyes are introduced into intact plants; i. e., into a closed system.

In this method a hole is bored into the trunk of a tree to a desired distance. The tapered threaded brass tube¹ devised for manometric studies in this laboratory is then screwed into the outer portion of the bore. The tapered part of the tube is screwed into the wood so firmly that it seals off the portion of the wood adjoining the outer part of the bore. A tubular glass reservoir is attached by means of rubber tubing to the free outer end of the brass tube. When a hand-pump producing a pressure of 1 to 2 atmospheres is attached to the reservoir, dye may be forced through the tube into the bore-hole. Although a portion of the trunk is opened by this means, there is a tangential movement of the dye into adjacent unopened conducting tracts. As far as the distribution of the dye is concerned, the same pattern obtains when dyes are moving through unopened or opened systems.

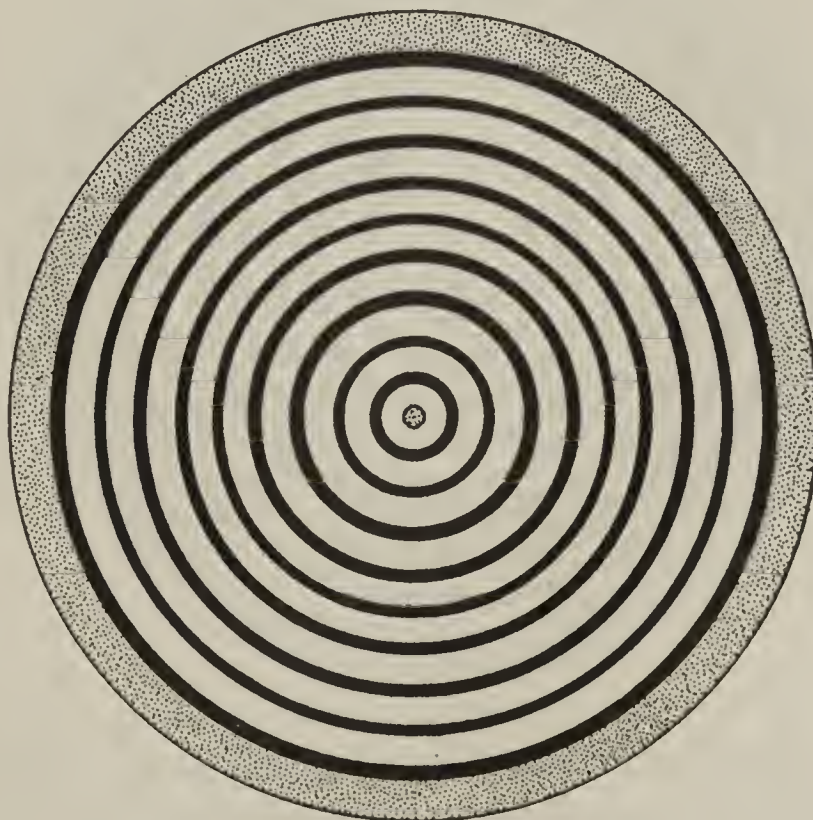
Botanists have long been in agreement that the only elements through which the transpiration stream moves are the vessels and tracheids of the xylem, and that those elements containing tyloses or gums do not function in conduction. As is well known, there is also great variation from genus to genus in the distribution of conducting and non-conducting elements. The anatomical organization of the stem may, therefore, affect the localization of the transpiration stream in the xylem; for example, the Monterey pine (fig. 8, page 29) has, except for the medullary rays, a secondary xylem composed

¹ MacDougal, D. T. The hydrostatic system of trees. Carnegie Inst. Wash. Pub. No. 373, p. 28, fig. 86. 1926.

exclusively of water-conducting elements. On the other hand, the walnut (fig. 6, page 25) has vessels more or less insulated laterally by wood fibers and xylem parenchyma. In the oak (fig. 7, page 27) the conducting elements are in radial sheets, which are insulated laterally by even broader sheets of non-conducting elements. Thus, as far as the distribution of the conducting elements is concerned, the transpiration stream may move through the entire body of secondary xylem or only through a portion of it. Our experiments soon demonstrated that all parts of these possible paths of conduction were not utilized to the same extent, and that it was not possible to predict *a priori* by microscopical examination whether all or only certain vessels would function.

When willow stems are cut off and stepped in dye, the leaves being allowed to transpire, the dye rises through practically every annual layer. This condition obtains in cut branches, in young trees, and also in large trees. An example of conditions in a large tree is shown by an experiment performed on June 16, 1926. An 11-year-old willow 10.5 m. in height, and 12 cm. in diameter at the base, was stepped in a tub of acid fuchsin and allowed to transpire for three days. Transverse sections cut at various levels from top to bottom of this tree showed that there was a conduction in all annual layers. Experiments on still older trees show that similar conditions obtain in all annual layers.

FIG. 11—Diagram of distribution of the transpiration stream (in black) in the various annual layers of *Salix lasiolepis*. Note that the transpiration stream passes only through the summer wood of each annual layer.



Although all annual rings in stems and branches of the willow show a conduction of dye, the various vessels of each annual layer possess unequal conducting capacities, and at least under midsummer conditions conduction is only in the later summer wood. This restriction of the dye to the late summer wood produces a pattern of concentric colored and uncolored rings on the transversely cut face of the trunk. (Fig. 11.) Irrespective of the width of the annual ring

the width of the colored zone in the late summer wood is less than a millimeter. The uncolored portion varies according to the thickness of the annual layer. In vigorously growing trees the uncolored zone is 2.5 to 4.5 mm. in thickness.

It might at first hand appear that the above-described zonation pattern is in some way connected with the opening of the system, and that there is not a zonation in an intact tree. That this is not the case is evidenced by results obtained by the injection of dyes into bore-holes by the method described on page 34. Injection of acid fuchsin under pressure into a 14-year-old willow showed at some distance above or below the bore-hole, the same zonation pattern as when cut stems are immersed in dye. The same zonation pattern also appears when the pull of a vacuum-pump is substituted for that of transpiration. It apparently makes no difference in the final distribution whether the dye is introduced at a cut end or by injection, or whether movement through the stem results from transpiration or from the pull of a vacuum-pump. A final zonation results in every case, conduction being mainly confined to the late summer wood, and none, or very little, occurring in the spring wood.

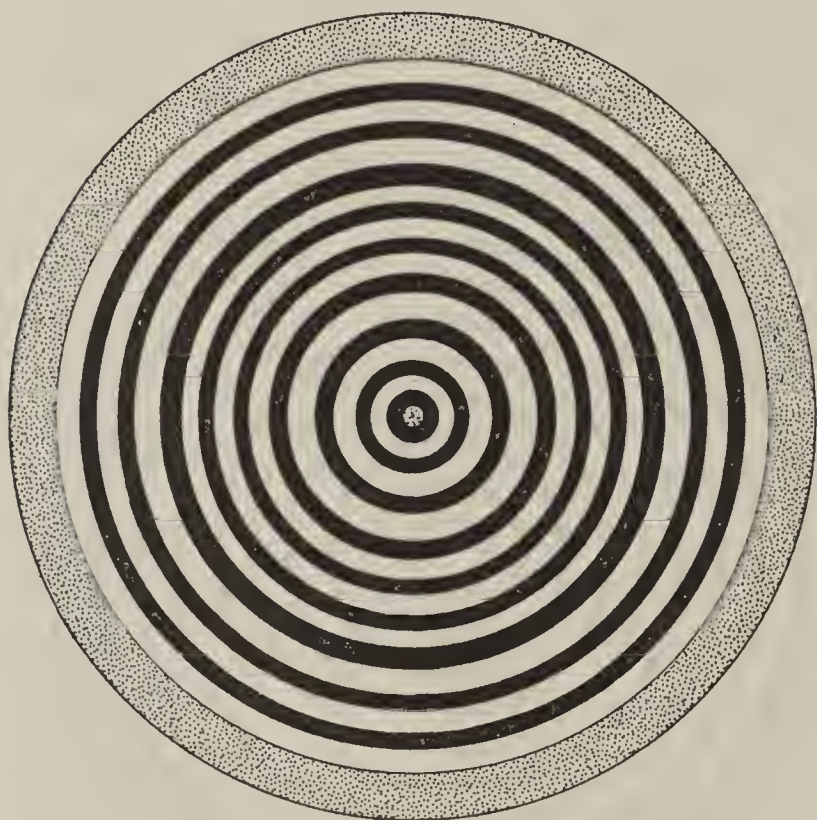


FIG. 12—Diagram of the distribution of the transpiration stream (in black) in the various annual layers of *Alnus oregona*. Note that the transpiration stream passes only through the spring wood of each annual layer.

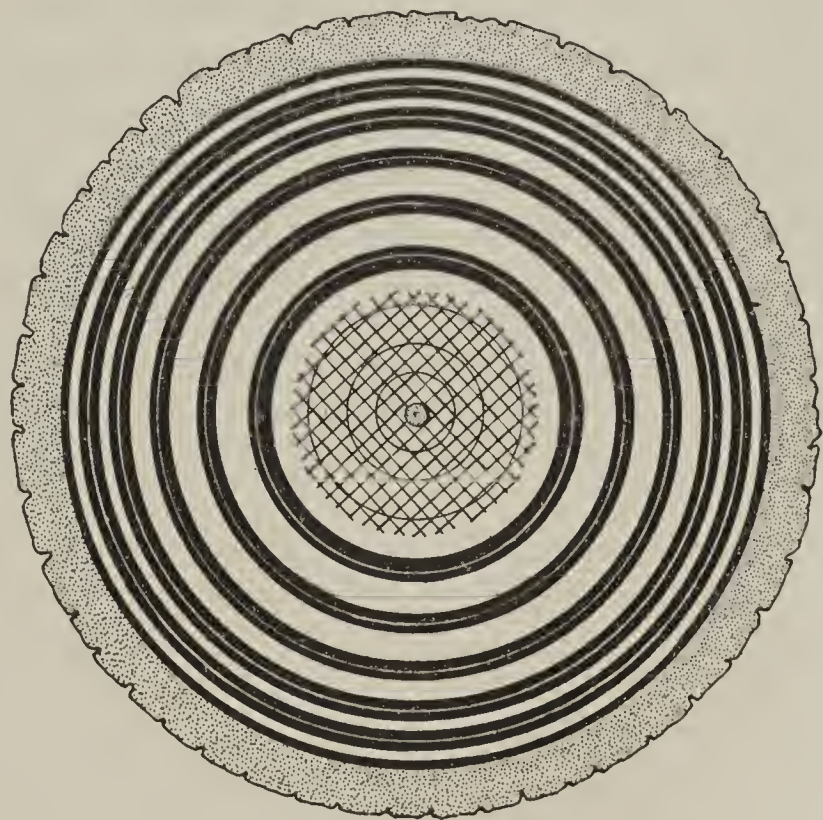
The strict localization of the transpiration stream to the late summer wood of each annual ring can not be determined with certainty when sections are cut and examined microscopically immediately after the tree is felled. Accurate determination of the elements concerned must be made by using sections of dried material mounted in paraffin oil, as described on page 33.

The previous statement that all possible paths of conduction are not utilized to the same extent becomes evident when similar experiments are performed on the alder and the walnut. In the alder there

is the same concentric zonation of the dye as in the willow, but the concentric colored zones are somewhat thicker. However, microscopical examination brings out the fact that the conducting portion of each annual layer is quite different in position from that in the willow. In the alder conduction is exclusively confined to the early-formed spring wood; there is no movement of the dye through the late summer wood. (Fig. 12.)

The path of the transpiration stream in the walnut differs from that found in either the willow or alder. As is well known, walnut trunks show a differentiation into sap-wood and heart-wood. The metamorphosis of sap-wood into heart-wood involves, among other things, a complete blocking of all vessels by tyloses. It follows, therefore, that conduction is only through such vessels as are devoid of tyloses. Such differentiation into heart- and sap-wood is of course found in many other genera than the walnut. The number of conducting layers at the base in trees of different genera but of the same age is not necessarily the same. The conducting belt may be a cylinder of many annual layers in thickness or, as in the chestnut¹ (*Castanea*), confined to the outermost layer only.

FIG. 13—Diagram of the distribution of the transpiration stream (in black) in the trunk of *Juglans californica*. The cross-hatched annual layers at the center of the trunk are tylosed. Note that there is no movement through the tylosed portion of the trunk, and that the transpiration stream passes through the late summer wood and early spring wood of each untylosed layer.



A topographical survey, based on a series of sections cut at various levels of a 12-year-old walnut stem, shows that for the first three or four years there is a tylosing of vessels in the spring wood of the current or of the previous year. Stems four or five years old have no tyloses in the two outer annual layers. At lower levels on the stem the number of untylosed annual layers is greater and becomes progressively larger toward the base of the tree. At the 12-year level, for example, there are no tyloses in the outer five annual layers; the

¹ Rumbold, Caroline. Effect on Chestnuts of substances injected into their trunks. *Am. Jour. Bot.*, 7: 45-56. 1920.

remaining annual layers are tylosed. It follows, therefore, that the conducting belt of xylem in the stem becomes progressively broader toward the base of the stem. As far as the individual conducting annual layers are concerned, conditions are different from those in either the willow or the alder. In each conducting annual layer of the walnut the dye moves through the outer portion of the late summer wood and the innermost portion of early spring wood, leaving an intermediate zone through which there is no conduction. (Fig. 13.) The zones of coloration are not as clearly defined as in the willow and alder.

When young trunks of the Monterey pine (*Pinus radiata*) are stepped in solutions of acid fuchsin, the dye first travels up in the three or four annual layers immediately within the cambium, and there is no coloration of the interior layers. Later the dye travels in the inner annual layers also, and all layers of the stem show coloration. In the case of the pine there is no localization of the dye to any particular portion of each annual layer, and, consequently, no sharp zonation as found in the willow, walnut, and alder.

The four cases above described show that the path of the transpiration stream through the xylem is not the same for all trees. In the pine the stream moves through practically all parts of each annual ring; in the willow it moves through the late summer wood only of each annual layer; in the alder through the early spring wood only; in the walnut it moves through the early spring wood and late summer wood of such annual layers as have not been transformed into heart-wood.

THE FACTORS AFFECTING THE LOCALIZATION OF THE TRANSPIRATION STREAM WITHIN THE CONDUCTING LAYERS

The marked localization of the transpiration stream in definite portions of the conducting annual layers, which has been described in the previous section, does not appear to have been stressed by any previous investigator. Strasburger¹ apparently observed a similar zonation, but his attention was so centered upon the question of the relative conductivity of spring and summer wood that he did not realize the significance of the phenomenon. The question of relative conductivity between spring and fall wood rests upon the size of the vessels, the nature of the intercommunications, and possibly upon the connection of the spring and summer wood with the leaves. This question of relative conductivity is not particularly applicable to the problem of zonation. In zonation one is dealing with conducting and non-conducting portions of annual layers.

The investigations by MacDougal² on the composition of gases in tree-trunks would appear at first glance to be wholly unrelated to the question of zonation of the transpiration stream. The fact that gases are present in trunks of trees has been recognized for a long time. Since the classical experiments of Schwendener³ and of Strasburger⁴ it has been generally accepted that the vessels and tracheids of woody plants contain not only water but also gas. The older view as stated by Copeland⁵ (page 174) is as follows:

“With the exception of a time in spring when the lumen of some trees becomes full of water forced there by root-pressure, the tracheæ *always*⁶ contain a considerable amount of air.”

There have been experiments to show that the presence of gases in the conducting tracts directly affects the volume of water transferred through the stem. For example, Hansen⁷ found that when the basal ends of severed branches were dipped in boiling water subsequent

¹ Strasburger, E. Ueber den Bau und Verrichtungen der Leitungsbahnen in den Pflanzen. Histol. Beitr., 3: 594-595. 1891.

² MacDougal, D. T. The hydrostatic system of trees. Carnegie Inst. Wash. Pub. No. 373. 1926.

———. Composition of gases in tree-trunks. Carnegie Inst. Wash. Year Book, No. 25: 160-162. 1925-26.

———. Composition of gases in trunks of trees. *Ibid.*, 26: 162-163. 1926-27.

———. Gases accumulating in tree-trunks. *Ibid.*, 27: ined. 1927-28.

³ Schwendener, S. Untersuchungen über das Saftssteigen. Sitzber. Preuss. Akad. Wiss., Jahrgang 1886: 561-602. 1886.

⁴ Strasburger, E. Ueber den Bau und Verrichtungen der Leitungsbahnen in den Pflanzen. Histol. Beitr., 3: 1891.

⁵ Copeland, E. B. The rise of the transpiration stream; a historical and critical discussion. Bot. Gaz., 34: 161-193, 260-283. 1902.

⁶ Italics are ours.

⁷ Hansen, A. Ein Beitrag zur Kenntniss des Transpirationsstromes. Arb. Bot. Inst. Würzburg, 3: 305-314. 1886.

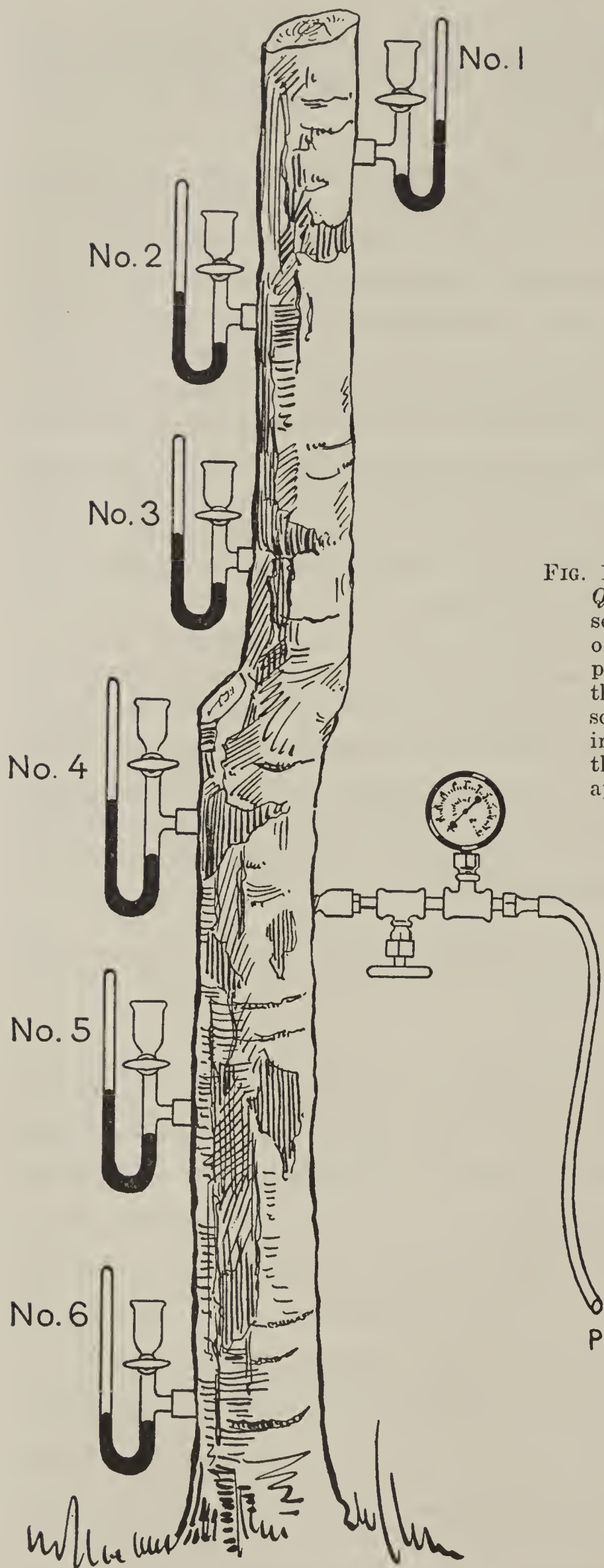


FIG. 14—Sketch of a trunk of a tree of *Quercus agrifolia* with manometers inserted to record vertical transmission of gaseous pressures. Pressures applied through the tube *P*, and entering the trunk through a tapered brass tube screwed into the trunk, are recorded in manometers both above and below the level at which the pressures are applied.

conduction was accelerated, a fact which Janse¹ showed to be due to the driving out of the air which partially blocked the vessels. Ewart² points out that in many cases the mere injection of dead wood with water may temporarily restore its conductivity so long as the continuous columns of water capable of transmitting tensions are maintained in the vessels; and states that the contention of Strasburger that the conductivity may be restored to dead wood by the injection of water is correct, but that the restoration is only temporary, the conductivity being rapidly lost under moderate suction by the entry of air.

Although the possibility of communication between gas-filled elements of the trunks has been recognized,³ no attempts have been made to determine the extent of such communication in living trees. Accordingly, on August 31, 1927, an oak tree (*Quercus agrifolia*) about 7 meters high, and 20 cm. in diameter at the base, was tapped a little over a meter above the ground, and into the bore-hole was screwed the special brass tube, as before described (page 34). Six closed manometers were attached to brass tubes inserted in bore-holes in vertical alignment at various levels above and below the tap, the highest being 210 cm. above and the lowest 80 cm. below the tap. Anticipating the probable need for a considerable pressure to affect the manometers remote from the bore-hole, the tube in the bore-hole was connected to a Stanley steamer capable of producing 600 pounds steam pressure. Steam under pressures varying from 20 to 25 pounds was applied for a few seconds only. (See fig. 14 for set-up.) That the pressures were rapidly transmitted up and down the tree was manifest by the behavior of the manometers, the one nearest above and the ones nearest below the tap responding almost instantly, and the others more slowly. It is evident that the pressures both upward and downward are transmitted rapidly and far beyond the vessel-lengths, which in this oak are 33 to 35 cm.⁴ The exact results of this experiment appear below in tabular form.

September 1, 1927

10^h 18^m a. m. *First application of steam*

Manometer coming up with jumpy motion, especially in

No. 3. Sap coming out of stem.

Steam shut off, pressure falling slowly.

10^h 22^m a. m. *Second application—15 lbs. pressure*

At 10^h 25^m No. 3 was minus 5.

¹ Janse, J. M. Die Mitwirkung der Markstrahlen bei der Wasserbewegung im Holze. Jahrb. Wiss. Bot., 18: 1-69. 1887.

² Ewart, A. J. The ascent of water in trees. Phil. Trans. Roy. Soc. London, B 199: 341-392. 1908.

³ Bailey, I. W. The structure of the bordered pits of conifers and its bearing on the tension hypothesis of the ascent of sap in plants. Bot. Gaz., 62: 133-142. 1916.

⁴ The lengths of vessels in the oak, walnut, willow, and alder have been determined by the use of oxyferric chloride solutions and by other means.

September 1, 1927—(Continued)

10^h 25^m a. m. *Third application*—20 lbs. pressure
Jerky rise of column No. 3.

10^h 28^m a. m. *Fourth application*—25 lbs. pressure

Pressures were applied for a few seconds only and fell to zero within 3 minutes. In manometers 3, 4, and 5, pressures fell quickly after the steam was shut off, but in No. 1 and No. 2 the pressure was maintained for some time.

Manometric pressures calculated in atmospheres are tabulated below.

First application at 10^h 18^m a. m.

No. 1.....	0.0	atmosphere
2.....	0.0	do.
3.....	0.0	do.
4.....	0.98	do.
5.....	0.46	do.
6.....	0.016	do.

Second application at 10^h 22^m a. m.

No. 1.....	0.018	atmosphere
2.....	0.014	do.
3.....	0.018	do.
4.....	0.26	do.
5.....	0.34	do.
6.....	0.016	do.

Third application at 10^h 25^m a. m.

No. 1.....	0.018	atmosphere
2.....	0.016	do.
3.....	0.018	do.
4.....	0.32	do.
5.....	0.19	do.
6.....	0.016	do.

Fourth application at 10^h 27^m a. m.

No. 1.....	0.018	atmosphere
2.....	0.016	do.
3.....	0.003	do.
4.....	0.091	do.
5.....	0.21	do.
6.....	0.016	do.

The introduction of steam into the trunk might possibly bring about deleterious effects on the tissues.¹ Since the above-described experiment showed that the anticipated high pressures were unnecessary, it was thought best to apply air-pressure by means of a hand-

¹It is of interest to note that this oak apparently experienced no injurious effects from the treatment with the steam. At the time this is written—twelve months later—the leaves appear perfectly normal. Undoubtedly many of the living cells of the trunk were killed by the treatment.

pump. A constant air-pressure was maintained by means of an automobile tire valve-stem inserted into the tube leading from the pump to the brass tube screwed into the bore-hole. The impulses from the pump showed pressures of 2 to 3 atmospheres on a gauge attached to the leading tube.

The general results obtained by increasing the internal pressure of the tree by the introduction of air into the bore-hole are comparable with those obtained by means of steam. It is therefore unnecessary to describe the results in detail. It should be noted, however, that with both steam- and air-pressures, all manometers do not register uniformly. When steam was used, these variations might perhaps be ascribed to the condensation of the steam. Similar variations were noted when air was forced into the trunk. The manometers near the bore-hole recorded pressures approximately equivalent to those introduced, while those at a distance recorded lower pressures, which were, however, still of considerable magnitude. For example, the manometer placed highest up the tree, 210 cm. from the receiving bore through which air was forced into the trunk, recorded a maximum pressure of two atmospheres. Such pressures are not long maintained and tend to fall slowly to the normal barometric pressure. It should also be noted that manometers approximately equidistant above and below the bore-hole do not record equal pressures, those above recording higher pressures than those below.

Similar experiments to those just described on the oak were performed on certain other trees. On September 7, 1927, a 14-year-old willow (*Salix lasiolepis*), 10 meters high, and 15 cm. in diameter at the base, was tapped 55 cm. above the ground. The bore-hole was 7 cm. deep and 5 mm. wide, tapping all annual layers except the four outside ones which were sealed off by the brass tube inserted into the bore-hole. On this tree closed manometers were placed in vertical alignment with the bore-hole, one 1 meter above and the other 4.75 meters above the bore-hole. A hand-pump was attached to the brass screw-tube in the bore-hole. With the first impulse of the pump, not over 1 atmosphere, a pressure of 25 mm. of mercury was shown immediately in the manometer nearest to the bore-hole and inside of 1 minute a pressure of 10 mm. of mercury in the manometer at the 4.75-meter level. The experiment was repeated, using a pressure of about 1.3 atmospheres in the hand-pump with similar results. As already stated, the length of the vessels in the willow are 7 to 10 mm. The pressures were quickly transmitted for a distance of 4.75 meters or far beyond the length of a single vessel. It should be noted that in the willow the vessels are much shorter than in the oak, and yet that the pressures were quickly transmitted in both cases.

On August 22, 1927, a two-forked red alder tree (*Alnus oregona*), about 12 meters high and 15 cm. in diameter at the base, was treated

similarly to the above-described willow tree, the bore-hole to which the hand-pump was attached being driven in 50 cm. above the fork. A manometer was placed in vertical alignment with and above the bore-hole at 1 meter. Under a pressure from the hand-pump of 1 to 3 atmospheres no change of pressure was manifest in the manometer for some time, after which there was a rise of 2 mm. of mercury. Injection of air was very difficult. Another manometer was placed midway between the tap and the one already inserted. When air was injected into the tree under pressure this manometer responded immediately, the mercury rising to 3 mm. in 2 to 3 minutes and to 10 mm. in 5 minutes. It will be noted that the gas was forced with more difficulty into the alder than into the oak or willow. In the alder the vessels are comparatively short, being only 7 to 8 cm. long. In this case the gas under pressure had to pass more septa than in the willow, but it is evident that length of vessels is not the sole factor governing the distance to which pressures are transmitted in the two trees.

About the middle of September, 1927, a small redwood tree (*Sequoia sempervirens*), 10 to 12 cm. in diameter at the base, was treated in a similar manner. The tree was tapped 1 meter above the base, and a manometer placed in line with the tap 1 meter above the tap. Air was forced into the tree more easily than into the alder, but no change in the manometer was evident after 10 minutes. The manometer was moved down to 0.50 meter above the tap, and pressure of 1 to 2 atmospheres again applied with no change in the manometer after 10 minutes. The manometer was then placed 0.25 meter above the tap, and air again injected with no change in 10 minutes. Using the same set-up, an attempt was made to inject fuchsin into the same tree, but no reliable result was obtained. Apparently, for a reason discussed elsewhere, neither air nor liquid moves with readiness through the tracheids of the redwood with the small pressures used in the experiment.

From the results of the above-described experiments it seems evident that when gases are forced into the trunks of trees under a pressure of 1 to 2 atmospheres, the rapidity and magnitude with which the pressures are transmitted is directly related to the length of the conducting elements. For example, in the oak, as already recorded, the vessels are 33 to 35 cm. long. Assuming that vessels lie end to end in a vertical file, this would mean about ten end-walls to be traversed in a vertical transmission of 3 meters. A gas-pressure transmitted the same distance through a willow stem would have to pass through 30 to 42 end-walls of vessels; in the alder 37 to 42 cross-walls; while in the redwood 650 to 1,000 end-walls would have to be passed. Although the length of conducting elements is an important factor in transmission of gaseous pressures, it is not the sole deter-

mining factor, because structural characters of pits between vessels and tracheids also affect the transmission of pressures.

Although gaseous pressures in trees are relatively easily and rapidly transmitted vertically, our results agree with those of Jones et al.,¹ that pressures are transmitted radially, if at all, with great difficulty, and that the hydrostatic system and the gaseous system are in connection up and down the stem more than radially. This was demonstrated by taking a portion of a 20-year-old living willow trunk, 35 cm. long and 11 cm. in diameter, and arranging it so that a suction of about one atmosphere could be applied at the top on a given annual ring. This was accomplished by screwing a small valve-stem of an automobile tire into the layer of wood to which suction was to be applied. Another valve-stem was screwed into the same layer at the bottom of the log, but tapping the same annual layer on the opposite side from the tap at the top. A glass tube with its end immersed in water or dye was attached to the lower valve-stem. The whole log was thoroughly covered with a heavy grease to prevent the entrance of the outside air. When suction was applied at the upper tap, 15 c. c. of sap was extracted in 2 hours, but the water rose only to a height of 4 cm. in the tube attached to the lower tap after the application of suction for 8 hours, showing that no gas passes radially or tangentially around or across this stem. If any is so transmitted the amount is very small.

In another experiment a 20-year-old willow log was so arranged that the taps were diametrically opposite each other, but tapped the same annual layers of wood. After several hours of suction no gas was drawn across or around the stem. The apparatus then was so arranged that the two taps were at an angle of 45 degrees apart in the same annual ring. When suction was applied for 2 hours, no gas was drawn across or around the stem. The angle between the taps was then reduced to 22.5 degrees. When suction was applied in this case a small amount of gas passed around the stem in 10 minutes, as was evidenced by a slight rise of the liquid in the glass tube.

Experiments were performed using the same method as above described, but substituting pressures for suction. In all cases the results were negative, except in those where the angle between the taps was not more than 22.5 degrees.

The foregoing experiments on the transmission of gaseous pressures demonstrate that there is a very much more extensive and continuous pneumatic system within the xylem than has been previously recognized, but they do not shed any light on the localization of the gas-body to definite regions of the xylem. The experiments to be described presently *do show* definitely which portions of the xylem

¹ Jones, C. G., A. W. Edson, and W. J. Morse. The maple sap-flow. Vt. Agr. Exp. Sta. Bull., 103: 43-184. 1903.

contain gases and which portions contain water. They will also show that vessels and tracheids may contain exclusively either water or gas, and that those elements containing gas are not available for the conduction of the water of the transpiration stream. Therefore, the definite localization of the transpiration stream described on pages 35-38 is to be ascribed to the presence of gases in definite portions of each annual layer. The conducting portion of the xylem is not a solid, or, in the case of a tylosed stem, a hollow cylinder of water, but consists of a series of alternate cylinders of gas and of water.

The foregoing conclusions as to the distribution of gas and of water within the xylem are based upon a variety of experiments.

If a short piece of living willow stem is completely immersed in acid fuchsin, the penetration of the dye into the wood will result in the same pattern of concentric zonation as when dye moves through the stem by a transpirational pull or by that of a vacuum-pump. On the other hand, if a similar piece of stem is immersed in dye, placed in a vacuum-chamber and the air exhausted, the dye will penetrate all portions of each annual layer. The zonation of the dye observed in the first case results from the fact that vessels of the spring wood are gas-filled and water can not diffuse into them, while in the second case the gas in these vessels will be removed and replaced by the dye. The same is true if the gas in the vessels is removed by boiling a portion of the stem in dye.

It is also possible to demonstrate visually the localization of the gas to the spring wood. When a piece of living willow stem is placed vertically in warm water, gas-bubbles may be seen to come out of the vessels of the spring wood only, and to accumulate in rings on the cut face. The same phenomenon is evident when a piece of living willow stem is immersed in chloroform, ether, or alcohol. It is evident, therefore, that the vessels of the spring wood of the willow are gas-filled.

We have also been able to demonstrate experimentally the presence of gas in definite portions of each annual layer in intact trees. On September 8, 1927, a 13-year-old willow tree, 10 meters in height, and 15 cm. in diameter at the base, was selected for the following described experiment. A bore-hole 5 mm. wide and 7 cm. deep was made in the tree 55 cm. above the ground. The bore-hole penetrated all annual layers. A brass screw-tube was inserted into the bore-hole so as to seal off the outer three annual layers of wood, but not the inner ten layers. Into this bore-hole 100 c. c. of acid fuchsin (1 part to 100 parts of water) was injected under a pressure of 1 to 2 atmospheres, by the method described on page 34. It is worthy of note that, when a liquid is injected, no very great changes in pressures are seen in manometers as contrasted to the changes induced by the

injection of gas or of steam. After half an hour the tree was cut down and the distribution of the dye studied. (Fig. 15.) The dye traveled upward for a distance of 55 cm. in all parts of all annual layers, except the outer three which were sealed off by the brass screw-tube. From the level of the bore-hole up to the 55-cm. level the portion of xylem solidly filled with the dye is a vertical column 5 to 6 mm. in width, that is, slightly wider than the bore-hole. At the level of the bore-hole there is only a slight tangential movement of the dye. Upwardly from the bore-hole there is a tangential distribution of the dye in the late summer wood, which becomes progressively broader toward the top of the stem. At the top of the solid column of dye the tangential movement in the late summer wood is a sector about one-twentieth of the circumference of the stem. About a meter above the bore-hole the dye is in the late summer wood only, and fills about one-sixth of the circumference of each annual layer. Toward the top of the tree the summer wood of each annual layer is completely filled with dye. As in other cases where the dye is not allowed to reach the leaves, the rate of movement is not the same in all annual layers. The dye traveled to the greatest height in the late summer wood of the 7th to 10th layers, and highest in the 9th layer. During the half-hour after the dye was injected and before the tree

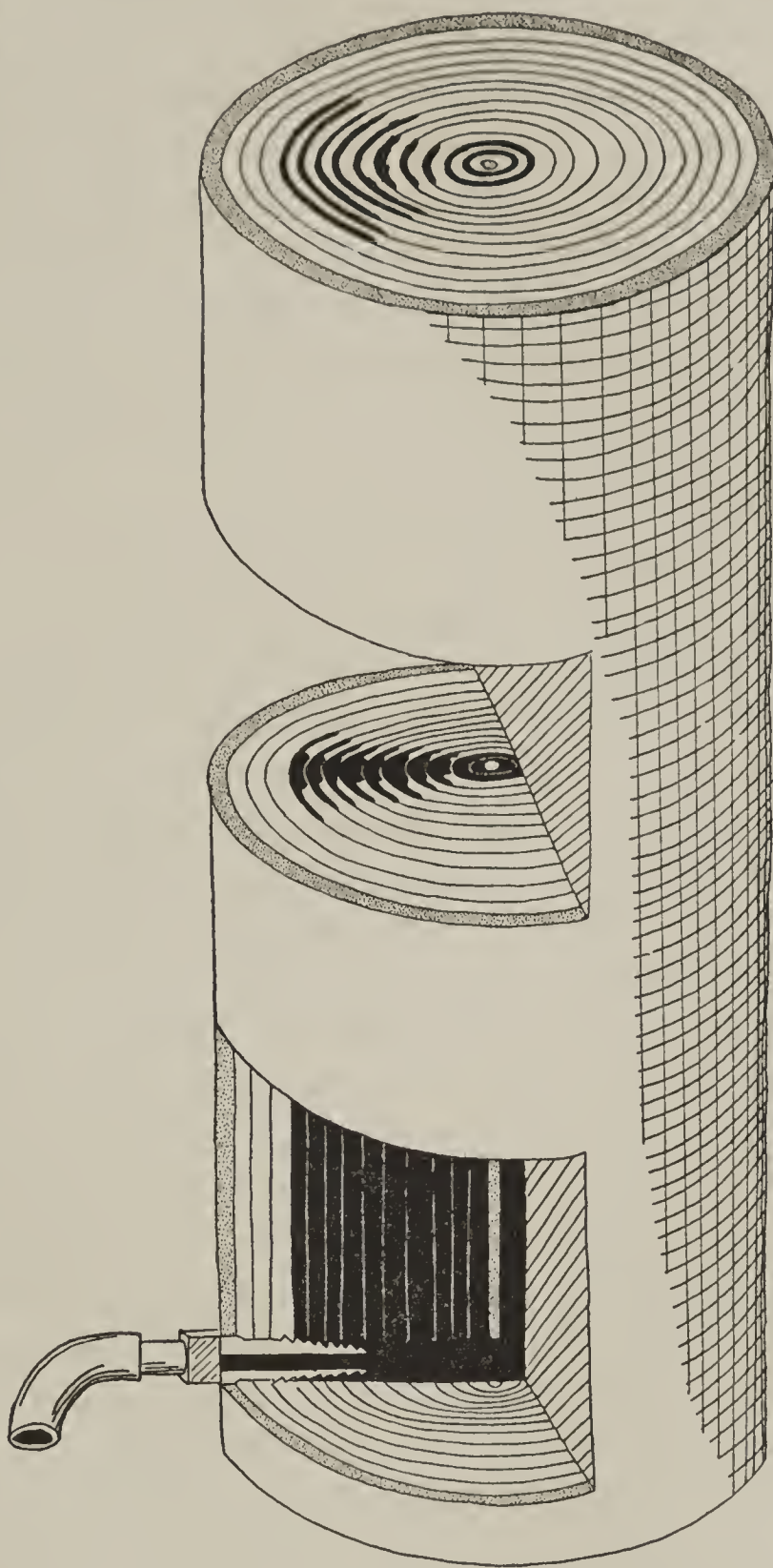


FIG. 15—Portion of a stem of *Salix lasiolepis* with sectors cut away to show the path followed by acid fuchsin injected through the tapered brass tube screwed into the bore-hole at the base of the stem. In the sector immediately above the bore-hole the dye moves through both spring and summer wood in the portions of the annual layers immediately vertical to the bore-hole. Higher in the stem the dye moves only through the late summer wood of these layers; that is, only through the portion of each annual layer through which the transpiration stream is passing. Note that there is some tangential movement of the dye as it passes upward from the bore-hole.

was cut the dye traveled in some layers to a distance of 1.5 to 2 meters.

It should also be borne in mind that the dye passes downward as well as upward from the bore-hole. In our experiments the same pattern of zonation obtains below the bore-hole as above it, the dye being present in the late summer wood only. In willow trees which have been injected in this fashion, the zonation pattern of the dye below the bore-hole has been traced downward to and into the root in those layers to which dye was introduced.

We interpret the presence of the dye in both spring and late summer wood for some distance vertically above and below the bore-hole as being due to the displacement of the gas in the spring and early summer wood by the dye. That the gas is thus displaced is shown by the fact that the distance to which the dye moves in spring and early summer wood is directly proportional to the amount of dye injected. When a small amount of dye is injected the distance to which the dye moves in the spring and early summer wood is limited, whereas the converse is true when a larger amount of dye is injected. The movement of the dye through the late summer wood appears to result from an establishment of contact between the dye injected into the bore-hole and the water-columns moving in the late summer wood. As a result of this establishment of contact at the open ends of the vessels of the late summer wood the usual zonation of the dye finally results. We are led to conclude from the above experiments that the localization of the transpiration stream in the willow to the late summer wood of each and every annual layer is due to the presence of gas in the vessels of the spring wood.

On September 22, 1927, a two-forked alder tree, about 12 meters high and 15 cm. in diameter at the base, was tapped 50 cm. above the fork. Fifty c. c. of acid fuchsin was injected into this alder tree, after which the tree was immediately cut down. Considerable resistance was experienced in the injection of the dye apparently for the same reason that gas was injected with difficulty. The distribution of the dye in the wood is very similar to that described for the willow. The dye passes only in the layers to which it has access, filling the whole of each layer at the level of the bore-hole and becoming zoned at a distance of 75 cm. above the bore-hole. Tangential movement occurs in each layer. Unlike the willow, the dye passes upward in the spring wood instead of in the late summer wood. That dye can only be forced into the alder with great difficulty is correlated with the fact that gas-pressures are transmitted with difficulty and for only relatively short distances (page 43). It furnishes additional proof that injection of dyes into non-conducting portions of annual layers is due to the displacement of gas, the gas in the alder being displaced with difficulty.

VARIATIONS IN THE PATH OF THE TRANSPIRATION STREAM

The statement appears in Benecke and Jost¹ that the vessels *always* contain water, and that, at certain times, as during growth of the vessels, and in trees during early spring, when root-pressure is vigorous, the vessels are often completely filled with water; but that, as soon as transpiration sets in, air is present in them. On the basis of the literature of the subject these authors conclude that this gas may enter the vessels as air and be dissolved in the water already in the root, or that it could diffuse into other portions of the plant and thence reach the vessels, where it would be dissolved in the water which they contain. They further state that, when transpiration begins, and the leaves require more water than the vessels can supply, a vacuum tends to be formed in the vessels, so that the dissolved air escapes in the gaseous form, a conception which is at variance with the findings of Bode.² Dixon³ holds that one of the most antagonistic criticisms of his theory of sap-flow is based upon the assumption that the water in the conducting tracts is broken by gas-bubbles.

It is stated by Benecke and Jost that air-bubbles in the water of the transpiration stream have a pressure less than one atmosphere, and that, therefore, they will act in a sucking manner, water being withdrawn from the neighboring vessels or tracheids, and that in them in turn air-bubbles under low pressure will appear. It is stated that the rarefaction of the air reaches its maximum during greatest transpiration; but it may disappear again entirely during the night owing to a continuous entrance of water through the root, while the vessels, when the air becomes dissolved once more, become full of water. It is held that if the rarefaction of the air continues for a longer period, air from the outside enters through the walls of the vessels. When the air-pressure within and without the vessel is by this means equalized, the vessels can not be again completely filled with water, and further entrance of air, in the long run, will interfere with the capacity of the wood for transporting water.

According to our observations, as recorded in the previous section, the vessels, which are operative in conduction, do not contain alternating gas-bubbles and water-columns in the form of a "Jaminian chain," but the vessels in the conducting annual layers contain exclusively either gas or water. While some vessels may perhaps contain water and gas in the form of a "Jaminian chain," they are not operative in water conduction. This statement, of course, should not be

¹ Benecke, W., and L. Jost. Pflanzenphysiologie. Vierte Aufl. Jena, 1924.

² Bode, H. R. Beiträge zur Dynamik der Wasserbewegung in den Gefäßpflanzen. Jahrb. Wiss. Bot., 62: 92-127. 1923.

³ Dixon, H. H. The Transpiration Stream. University of London. 1924.

taken to mean that gases may not be in solution in the water which completely fills the conducting tracts.

The assumption should not be made that the concentric stratification of the transpiration stream, which has been described in the previous section, is fixed, and that there are no variations in the proportions of gas-filled and water-filled elements. There is a marked seasonal variation in the width of the water-containing portion of each conducting layer. Our descriptions of the gas-filled and water-filled portions so far given are those of midsummer conditions, at which time the concentric zonation of the dye is most striking. The results obtained to substantiate this point in the case of the willow are not particularly conclusive, because at the close of this work in December many willow trees had not yet shed all of their leaves. We shall, therefore, confine our descriptions to conditions which obtain in the alder.

On December 1, 1927, a seven-year-old alder tree about six meters in height, and which still retained its leaves, was cut and the upper leafy portion stepped into acid fuchsin and allowed to stand for twenty-four hours. At this season of the year zonation of the dye is still evident, but the zone of conduction in each annual layer is very much wider than in summer, considerable conduction occurring in the interior of each layer. Suction experiments on portions of this tree show that the gases in the wood are nearly replaced by water. The volume of conduction at this season of the year is relatively high as compared with that in summer when the wood is largely gas-filled.

On December 27, 1927, a seven-year-old alder tree six to seven meters in height, which had completely shed its leaves, was cut, and a portion of the upper part was stepped in a solution of acid fuchsin and allowed to stand for some time. Zonation of the dye occurs in all annual layers, but transmission of the dye is not confined to the spring wood as is the case under midsummer conditions. In cross-sections of the stem the dye occurs in radial streaks in all parts of each annual layer between the spring wood and late summer wood. Suction experiments on portions of the same tree result in the dye being distributed in practically the same way as above described. In this case also the volume of conduction is relatively high as compared with that in summer. These experiments indicate that the gases which are present in all vessels of each annual layer, except those of the early spring wood, are being replaced by water. At this season of the year this wood is apparently approaching a condition in which the gas in the vessels is being replaced by water.

Another method of demonstrating the seasonal variation in the extent of the gas-body within the tree is the ease with which dyes can be injected into stems at different times of the year. We have not discussed injection experiments with the oak because of difficul-

ties in definitely determining which conducting elements contain water and which contain gas. However, *Quercus agrifolia*, the California live oak, is an especially favorable object for experimentation, because there is a marked seasonal variation in the ease with which dyes may be injected into the stem. On September 9, 1927, a fourteen-year-old stem of this species, 4 to 5 meters long and 14 cm. in diameter at the base, was tapped by a bore-hole 5 cm. deep. A brass screw-tube was inserted into the bore-hole, and through the tube 100 c. c. of acid fuchsin (1 part to 100 parts of water) was injected under a pressure of 1 to 2 atmospheres with very great ease.

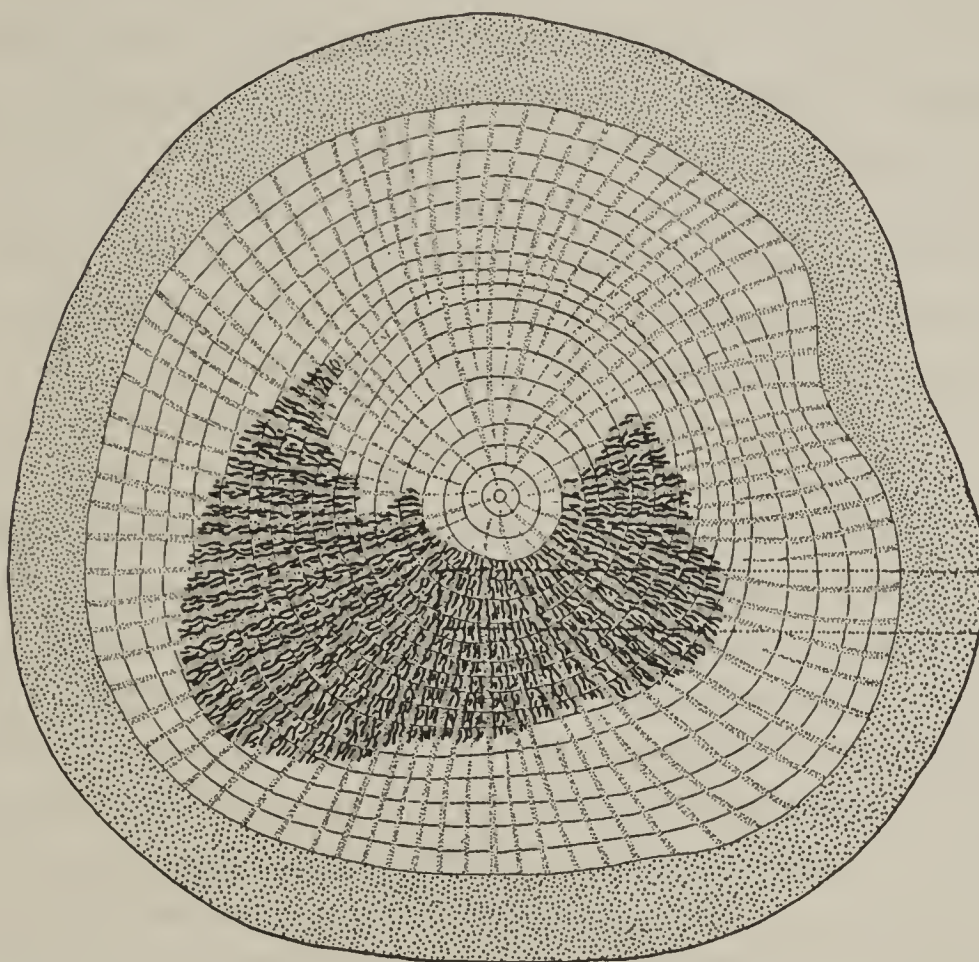


FIG. 16—Diagram of the distribution of acid fuchsin injected through a bore-hole into a stem of *Quercus agrifolia*. The regions through which the dye passes are shown in black: the bore-hole through which the dye was injected, and below the level at which the transverse section was cut, is shown by a dotted line.

The injected dye traveled upward and downward from the bore-hole for considerable distances. (Fig. 16 shows the distribution of the dye immediately above the bore.) On December 5, 1927, an oak tree of somewhat smaller dimensions was treated in a similar manner. Injection of the dye was extremely difficult when compared to that of September, and only 30 c. c. of dye could be injected into the stem. The differences of behavior in the two cases are ascribed to the fact that under midsummer conditions many of the conducting elements contain only gas, whereas at the beginning of winter most of these elements are water-filled.

As will be recalled from the experiment described on page 41, gaseous pressures, as indicated by air-filled manometers, are rapidly

transmitted up and down the stem. On December 5, 1927, an oak tree about 5 meters in height and 14 cm. in diameter at the base, was tapped 50 cm. above the ground. Closed manometers were attached to radially driven bore-holes, as in the experiment illustrated in fig. 14, page 40, one being 25 cm., one 50 cm., and the third 100 cm. above the tap. When an endeavor was made to force air into the tap under a pressure of 2 to 3 atmospheres, great difficulty was encountered in getting any air into the tree, and no response whatever was evident in any of the manometers. In the similar experiment performed on September 9, 1927, pressures introduced into an oak tree were quickly transmitted for considerable distances both up and down the tree. It would seem evident from these results that the difference in the transmissibility of pressures in September and December is due to the fact that in December many more of the vessels are filled with water than in summer, with a consequent displacement of gases.

It is generally admitted that in deciduous trees during winter in temperate climates the amount of gas in the wood is reduced to a minimum (resulting in so-called negative pressure) and that in some trees the wood fills up with water. Von Höhnel¹ has shown that "negative gas-pressures" gradually disappear when transpiration ceases, and supposes that the daily variations in the percentage of water acts directly in maintaining "negative pressure" in the vessels.

Our experiments have shown that there is a seasonal periodicity in the vacating and refilling of certain conducting elements in the neighborhood of those containing the continuous water-columns, which is conditioned among other things by a seasonal variation in the rate and amount of transpiration. When transpiration is active, the tensile columns of water in the conducting tracts are subject to variations, and some of the dissolved gases may come out of solution and may fill up some of the elements previously filled with water. There is also the possibility that, when water is abstracted from vessels and tracheids by transpiration at a more rapid rate than it can be supplied to them, gases may enter from neighboring gas-filled elements. As a result the vessels and tracheids which have become filled with gas no longer serve in the conduction of water. On the other hand, the reverse condition may obtain and a diminution of the tension on the water in water-filled elements might be followed by a refilling of the neighboring gas-filled elements with water, or by a total solution of the gases, and by an extrusion of the gases into intercellular spaces if such are present.

Although diminution in tension on the water in the conducting tracts is doubtless a contributing factor in the accumulation of water in trunks of trees at the end of the growing-season, when the leaves

¹ Von Höhnel, F. J. Beiträge zur Kenntniss der Luft- und Saftbewegung in der Pflanze. Jahrb. Wiss. Bot., 12: 47-131. 1879.

are shed and transpiration is thus reduced to a minimum, it is, however, possible that the expansion of the water may not result in a complete filling-up or replacement of the gas in the lumina of all vessels and tracheids, but that gas-bubbles may possibly be present in some tracts which contain water and thus block them. Whether partially or completely filled with water, when transpiration begins at the time the leaves are expanded, the results would be a withdrawal of water from some of the water-filled lumina and their reoccupation by gases.

Undoubtedly other factors besides transpiration, including temperature changes, cooperate in variations in the relative amounts of gas and of water in the conducting tracts. One of the contributing causes to the filling of the wood with water is held to be due to "root-pressure," which is held to force water into the lumina of the vessels especially in spring. Atkins¹ holds that the starch-containing parenchymatous cells which lie adjacent to the vessels secrete sugars into the water of the transpiration stream, thus raising the osmotic concentration of the solutions in the vessels. The greatest concentration of sugars occurs in early spring. With the advance of spring and early summer there is a rapid dilution of the sugars which reaches its maximum in late summer and early autumn. On the assumption that there is an increase in osmotic concentration of sap in vessels of roots, Atkins holds that there will be a flow of water from the soil to the vessels, so that the resulting hydrostatic pressure is responsible for the phenomena of bleeding and root-pressure characteristic of the spring. He also holds that the resulting hydrostatic pressure forces much air into solution, and removes the air-bubbles from the conducting tracts so that the volume of the transpiration stream is increased. Atkins, in common with certain other investigators, appears to hold that root-pressure is of major importance in the sap-pressures developed by trees in spring, and the consequent forcing of sap into the gas-filled portions of the plant. It is, however, evident that sap-pressure sufficient to cause this phenomenon may arise in other portions of the plant than in the root, as is evidenced by the work of Jones² and his coworkers, who show that sap-flow in the maple is limited to a very few weeks in spring, a condition which is ascribed to an excess of water absorption over water-loss. In early spring in Vermont a blanket of snow keeps the ground around the roots of the maple trees relatively warm, and the lengthening days raise the temperature of the trunks sufficiently to allow the stored carbohydrates to be converted into sugars, with a consequent rise in the osmotic concentration of the sap in the vessels, which makes possible

¹ Atkins, W. R. G. Some recent researches in plant physiology. London, 1916.

² Jones, C. G., A. W. Edson, and W. J. Morse. The maple sap-flow. Vt. Agr. Exp. Sta. Bull., 103: 43-184. 1903.

the absorption of large quantities of water, not much of which is transpired because the new leaves are not yet expanded. Therefore, external conditions both below and above the ground and internal conditions also result in a relatively high sap-pressure, which may result in filling the trunk with water. It may well follow that under these conditions the amount of free gas present in the trunk would be relatively small, but that as soon as transpiration begins and other factors, such as temperature changes, etc., modify this sap-pressure, some of the vessels may be emptied of water and replaced by gas.

It is commonly assumed that in summer, under the usual mesophytic conditions in temperate regions, when transpiration is active, positive internal pressures are entirely absent or very greatly reduced. Under the local conditions of a long rainless summer, during which we have been working, it is remarkable that in the willow and in the walnut positive pressures may be found only in tangential bores driven into the outer layer of trunks. During this midsummer season of slackened growth there are only suctions in radial bores, and at the ends of stumps of branches and roots.

It is not clear to what extent variations in pressures, as indicated by manometers, are due to changes in the hydrostatic system as differentiated from changes in the pneumatic system of the trunk. As previously shown, the hydrostatic system is, in effect, a series of concentric cylindrical columns laterally separated from one another by cylindrical columns of conducting elements, which are gas-filled.

We have investigated the live oak (*Quercus agrifolia*) especially to determine the interrelationship between the two phases of the tree's hydrostatic-pneumatic system. In one tree selected a water-filled manometer connected with a straight vertical tube standing in mercury showed suctions of about -80 mm. Hg. A small bore was driven into this tree and connected with a vacuum-chamber which could be exhausted to 750 mm. Hg. The vacuum-chamber was in continuous connection with the vacuum-pump so that a partial vacuum could be maintained continuously within the chamber. Air manometers attached to this trunk showed that suctions were transmitted to considerable distances along the gas system within the trunk, and that these suctions could be maintained for days by operating the pump from time to time. The effect of this reduction of the gaseous pressure within the trunk is to increase the suction on the hydrostatic system, and water-filled manometers register -155 to -175 mm. Hg., instead of around -80 . In a very broad way reduction of the internal pressure of the gaseous system is accompanied by an increase on the transpirational pull.

The effect of increase in pressure of the gaseous system upon the behavior of the hydrostatic system was also studied. Air-pressures of about 4 atmospheres applied at the bore-hole in the trunk were almost

immediately registered at nearly equivalent pressures in air manometers 2.5 to 3.5 meters above the bore. Such increases in the internal gaseous pressure within the trunk do not, however, have a corresponding effect upon manometers connected to the hydrostatic system, and there is no essential change in the suctions recorded by water-manometers. It is clear that varying pressures in the gas-body exercise only a minor influence on the tension in the cohesive water system.

THE PATH OF THE TRANSPIRATION STREAM: LATERAL MOVEMENT IN THE STEM

Since the leaves of the dicotyledonous trees under consideration are in connection with the current year's xylem through the leaf-traces, and since our experiments show that the transpiration stream moves vertically through the annual layers of previous as well as through that of the current year, it is necessary to consider the path by which the transpiration stream moves from the inner layers to the outermost one. On first thought one might consider the medullary rays to be the pathway for radial transfer of the water of the transpiration stream, but in the dicotyledons under consideration, as well as in all other dicotyledons, the medullary rays are composed exclusively, at least in the sap-wood, of living parenchymatous cells. Consequently there can be but little if any radial mass movement from the inner to the outer layer through the medullary rays. Such movements as do take place through the rays are largely or wholly osmotic. On the other hand, in certain gymnosperms such as *Pinus*, whose medullary rays contain tracheids, there exists the possibility of radial mass movement through the medullary rays.¹

Anatomical observation of stems of perennial plants shows that the last-formed vessels or tracheids of one year's growth may abut directly upon the first-formed vessels or tracheids of the succeeding year's growth. Strasburger,² having demonstrated the presence of pits between the adjoining elements formed in successive years, postulates the view that by this means there may be a radial transfer from one annual layer to another. To test the validity of this hypothesis, a portion of a freshly cut living willow trunk 22 cm. long was so arranged that a suction of slightly less than one atmosphere could be applied at the top of a given annual layer of wood. This was accomplished by screwing a small valve-stem of an automobile tire into the layer of wood to which suction was to be applied. (Fig. 17.) Another valve-stem was screwed into the same or into a different layer at the bottom of the log. The lower valve-stem was filled with and set in a solution of acid fuchsin, and the upper and lower ends of the log were securely sealed with hard grease. Suction was applied to the upper valve-stem by connecting it by pressure tubing with a rotary air-pump, which maintained a column of mercury 740 mm. in height.

When intake and outlet valves are in the same annual layer and vertically in line with each other, the dye moves from lower to upper

¹ MacDougal, D. T. Reversible variations in volume, pressure, and movements of sap in trees. Carnegie Inst. Wash. Pub. No. 365, p. 4. 1925.

² Strasburger, E. Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. Histol. Beitr., 3: p. 191. 1891.

valve-stem in less than one-half hour. A longitudinal dissection of such a log shows that the dye moves through the late summer wood only and through a vertical column of the same diameter as the opening of the valve-stem. The above-described experiment having demonstrated the speed with which dye may be moved through the stem, a second portion of similar length and diameter was taken from the same trunk, and the upper and lower valve-stems screwed into different annual layers but radially to each other. After suction had been applied for one hour the dye had moved upward less than 5 cm. through the annual layer connected with the lower valve-stem, and there was no radial transfer to outer layers.

Two explanations are possible for the failure to obtain radial transfer of the dye from one annual layer to another in the experiment just described. (a) The radial movement of dye is impossible because all vessels of the spring wood are filled with gas. (b) The pits between the abutting last-formed vessels of one year and the first-formed vessels of the following year, although anatomically demonstrable, are functionally inoperative. In order to determine whether or not gases in the spring wood are the major factor in prevent-

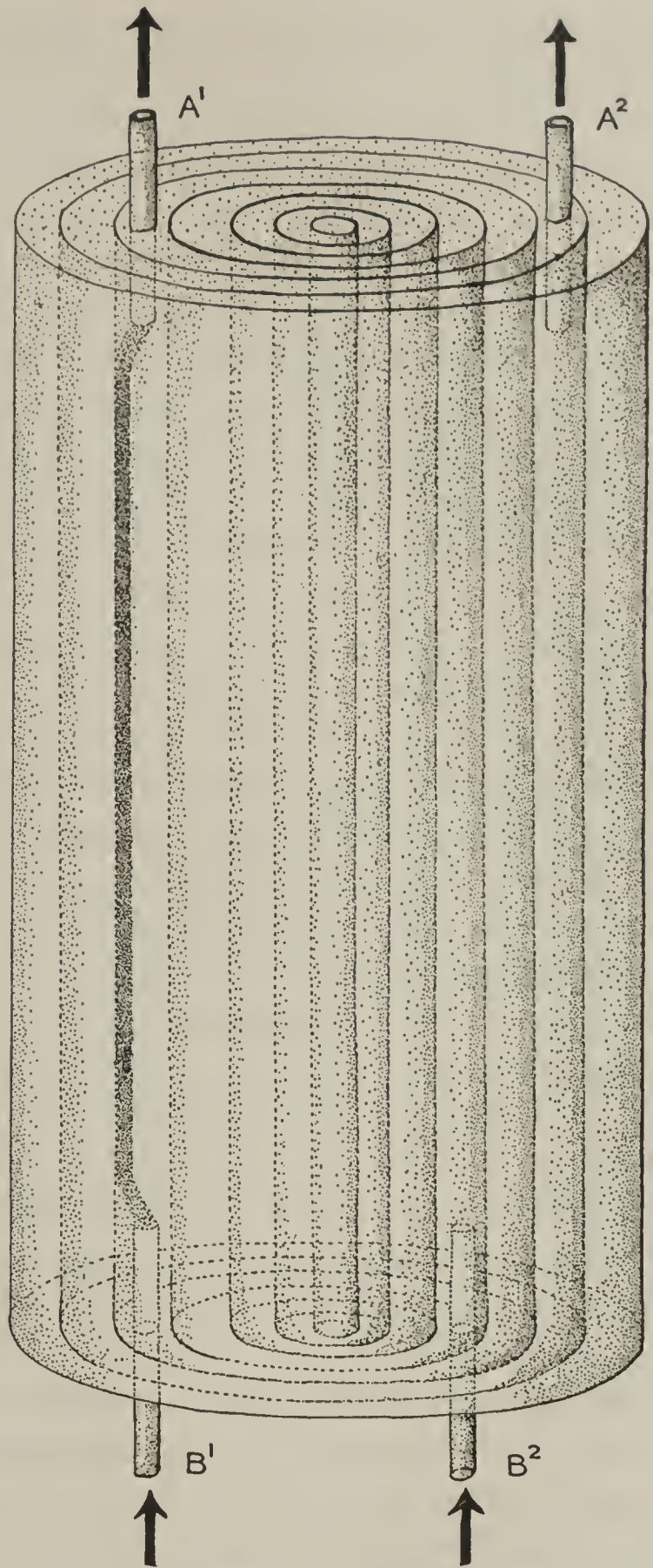


FIG. 17—An experiment designed to show radial movement of dyes from one annual layer to another in a trunk of the willow. The portion of the willow trunk used in the experiment is drawn as if the various annual layers were transparent. On the left-hand side of the figure the tap to which suction was applied (A^1) is vertically above, and in the same annual layer, as the intake tap (B^2) which stood in acid fuchsin. In this case there was a vertical movement of dye the length of the portion of the trunk. On the right-hand side of the trunk the suction tap (A^2) is vertically above, but radial to, and tapping a different annual layer, from that into which the intake tap (B^2) is screwed. In this case there was no upward movement of the dye.

ing radial movement the following described experiment was performed.

A portion of a 14-year willow stem 11 cm. in length and 6 cm. in diameter was submerged in water and placed in a vacuum-chamber, and evacuation continued until no more bubbles could be seen coming from the cut ends of the wood. In order to determine that the wood became infiltrated with water a similar portion of the same stem was immersed in acid fuchsin in the same chamber. Because the portion immersed in acid fuchsin showed a more or less complete penetration of the dye in all parts of all annual layers, it was assumed that in the log immersed in water under the same conditions the gases were removed from all the vessels and replaced by water. Taps were then inserted in the water-filled piece of wood so as not to penetrate the same annual layers, but to be radial to each other. Suction was applied for several hours after which the stem was dissected. The dye moved vertically in all parts of all layers directly above the intake-tube, or throughout five annual layers. The dye was also present immediately below the exit-tube and in portions of three or four annual layers external to those which the dye entered. This experiment demonstrates that radial transfer is possible in water-filled stems of the willow. The apparent limiting factor preventing radial movement of the transpiration stream under mid-summer conditions is the presence of gases in the vessels of the spring wood. Although all vessels may be water-filled, radial transfer is difficult in comparison with longitudinal movement, as is evidenced by the much greater rapidity with which dyes move between the intake and outlet taps in vertical alignment, as compared with the speed when the two tubes are vertical to each other, but radially placed.

Even more striking proof of the fact that there is no radial transfer from one annual layer to another in the willow is obtained from experiments in which dyes are introduced into certain inner annual layers and excluded from outermost ones. The method of injecting dyes into specific annual layers of wood has already been described on page 34. If, for example, dye is injected into a 13-year-old intact willow tree in such a manner as to enter the ten oldest annual layers, and be excluded from the three outermost ones, as shown in figure 15, page 47, it will be found that to the height of the 10-year level there is no dye in the outer three annual layers, to which the dye did not have access. There is of course a tangential movement of the dye in those annual layers to which the dye has access, and at a considerable distance above the bore-hole the tangential movement in the summer wood has been completely around the annual layer. The number of annual layers in which there is no dye remains constant from the bore-hole to the 10-year level; that is, the three outer layers

remain uncolored. On the other hand, the number of annual layers toward the center of the tree, which contain the dye, becomes progressively smaller until at the 10-year level (where there are four annual layers) only the innermost, or the terminal portion of the wood of the tenth year, carries the dye.

The experiments described above demonstrate that there is not a radial mass movement nor a radial transfer of the forces concerned in sap-flow from one annual layer to another through the living cells of the medullary rays or through abutting vessels. Although we maintain that there is no radial mass movement through the cells of the medullary rays from one annual layer to another, the possibility exists of an osmotic transfer of both water and solutes through these structures.

Although the injection experiments just described show that there is no radial mass movement from the inner to the outer annual layers, yet on examination leaves at the tips of all branches above the bore-hole show the presence of dye. Since these leaves are connected only with the wood of the current year, and there is no dye in this layer at the level of the bore-hole, there remains for consideration the path by which the transpiration stream moves from the inner layers to the outermost one. The problem may be stated in terms of a 2-year-old unbranched stem as follows: how does water move from last year's to the present year's wood if there is no radial mass movement through medullary rays, or radially through abutting vessels of the two years?

Possible paths by which the transpiration stream may move from the previous to the current year's wood are the leaf-traces. At the beginning of the second season of growth, as described on page 18, the leaf-traces of the previous year are present, although the leaves to which they led are no longer there. These old leaf-traces may, perhaps, be considered as possible paths of radial transfer of the water of the transpiration stream from the first to the second year's wood, unless structural changes in them prevent such radial movement. In the walnut the old leaf-traces develop tyloses in the vessels; no such obstructions have been found in the old leaf-traces of the willow. We have been unable to demonstrate the passage of water or dyes through these old buried leaf-traces from one annual layer to another in either the walnut or in the willow. We do not believe that the old leaf-traces offer paths for the radial transfer of the water of the transpiration stream, or for the transfer laterally or radially of tensions sufficient to account for the passage of water from layers internal to the current year's growth.

The two-year unbranched stem under consideration consists of a central column of xylem whose terminal end is bluntly conical. This central cylinder is surrounded by a thimble-shaped mass of wood

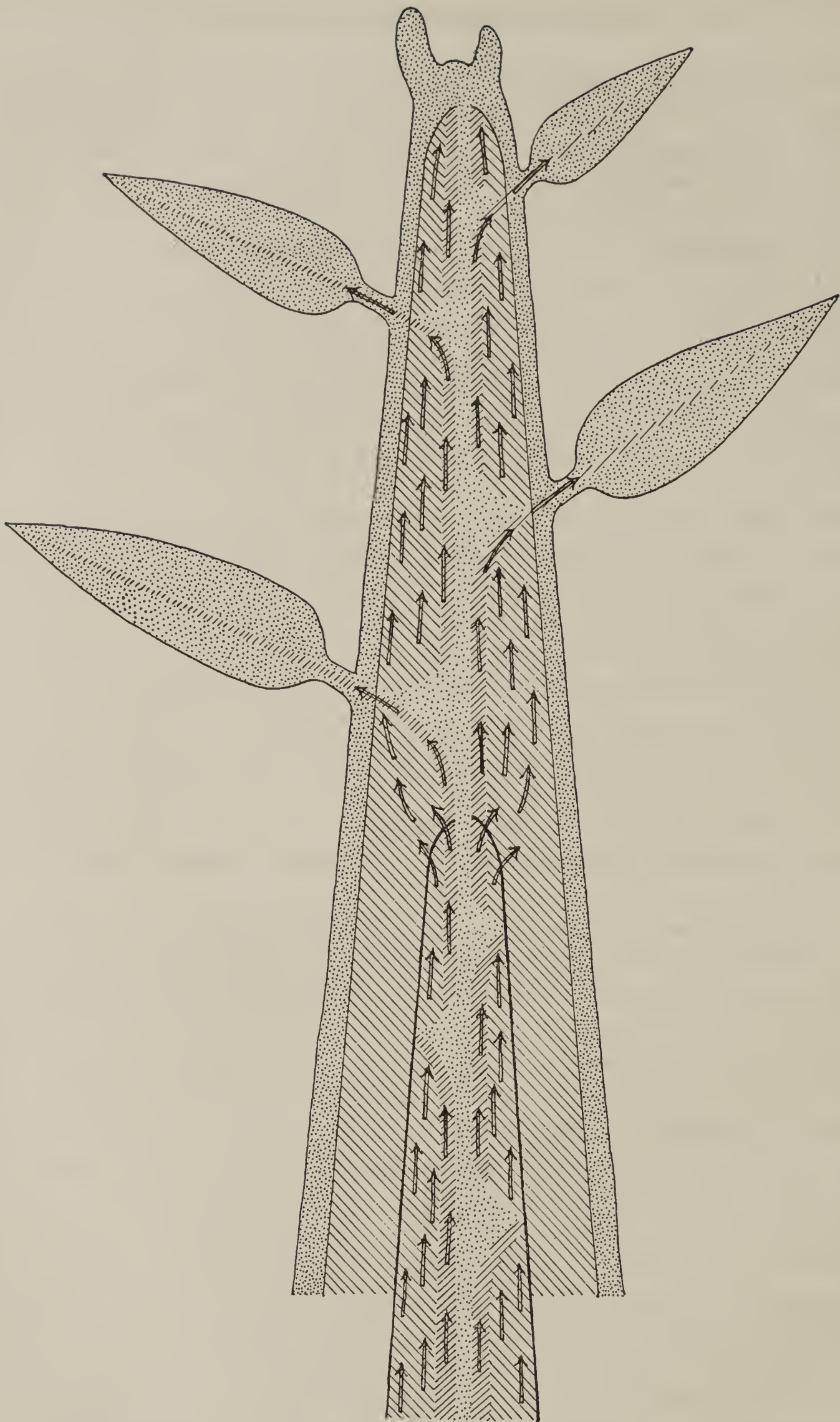


FIG. 18—Diagrammatic longitudinal section of a two-year-old, unbranched willow twig showing, by arrows, the movement of the transpiration stream from wood of the first year to that of the second year when water is coming into the stem through wood of the first year only. Primary and secondary xylem are diagonally shaded, in opposite directions; pith, phloem, and cortex are in stipple.

whose upper half (longitudinal growth of the present season) is solid, and whose lower half (the portion formed laterally to the previous year's wood) is hollow. Considering the distribution of the transpiration stream in this two-year-old unbranched stem, the majority of the vessels and tracheids of both primary and secondary xylem for some distance back of the growing apex contain water, i. e., the dome-like portion of the xylem at the apex of the stem, which caps but does not ensheath the preceding year's growth, contains water. (See fig. 18.) As the conducting elements of the leaves are in connection with those of the apex of the stem, the water-columns of this region can be acted upon by the transpirational pull exerted by the attached leaves. Since the secondary xylem of the first year caps and ensheathes that of the second year, the transpirational pull exerted by the leaves on the water in the elements of the second year may be transmitted to the water in those elements of the first year with which those of the second year are in contact. This contact is at the top of the dome-shaped mass of xylem of the preceding year, and it is here that the transpiration stream moves from the xylem of the first to that of the second year. It appears, therefore, that in the two-year-old twig water entering the first year's wood moves more or less vertically through it to the region where the second year's wood caps that of the first year.

The path of the transpiration stream from the innermost layer at the base of unbranched stems of more than two years old is essentially the same as in two-year-old stems. In stems older than two years annual layers of wood formed during the third and successive years have the same relation to one another as those formed during the first two years. That is, wood formed during any given year ensheathes and projects vertically above the previously formed cones of wood. Dye entering the innermost cylinder of such a stem passes longitudinally to the terminal portion of the cylinder and thence more or less vertically into the upper, solid, dome-shaped region of the succeeding year's xylem. After moving longitudinally through this xylem, which at this level constitutes the innermost annual layer, it moves in similar fashion vertically through the upper, solid, dome-shaped regions of all succeeding annual layers until it arrives at the terminal one which is directly connected with the leaves. Thus, dye starting up a stem through the innermost annual layer continues to travel up through the innermost layer only. It should be borne in mind, however, that these layers are not continuous in the sense that they were formed during the same year, but constitute consecutive additions to the stem.

The account just given is that of the effect of transpirational pull upon liquids entering a specific annual layer of the stem. In intact trees the transpirational pull is exerted on all annual layers, unless

these are blocked by tyloses, contain gases, or are otherwise inoperative in conduction. Our experiments on the walnut and willow show that not only the upper, solid, dome-shaped region of the first year's xylem is water-filled, but also that the upper similar portion of the secondary xylem of the second year's growth is water-filled. The same is true of the third year. The transpirational pull on the water-

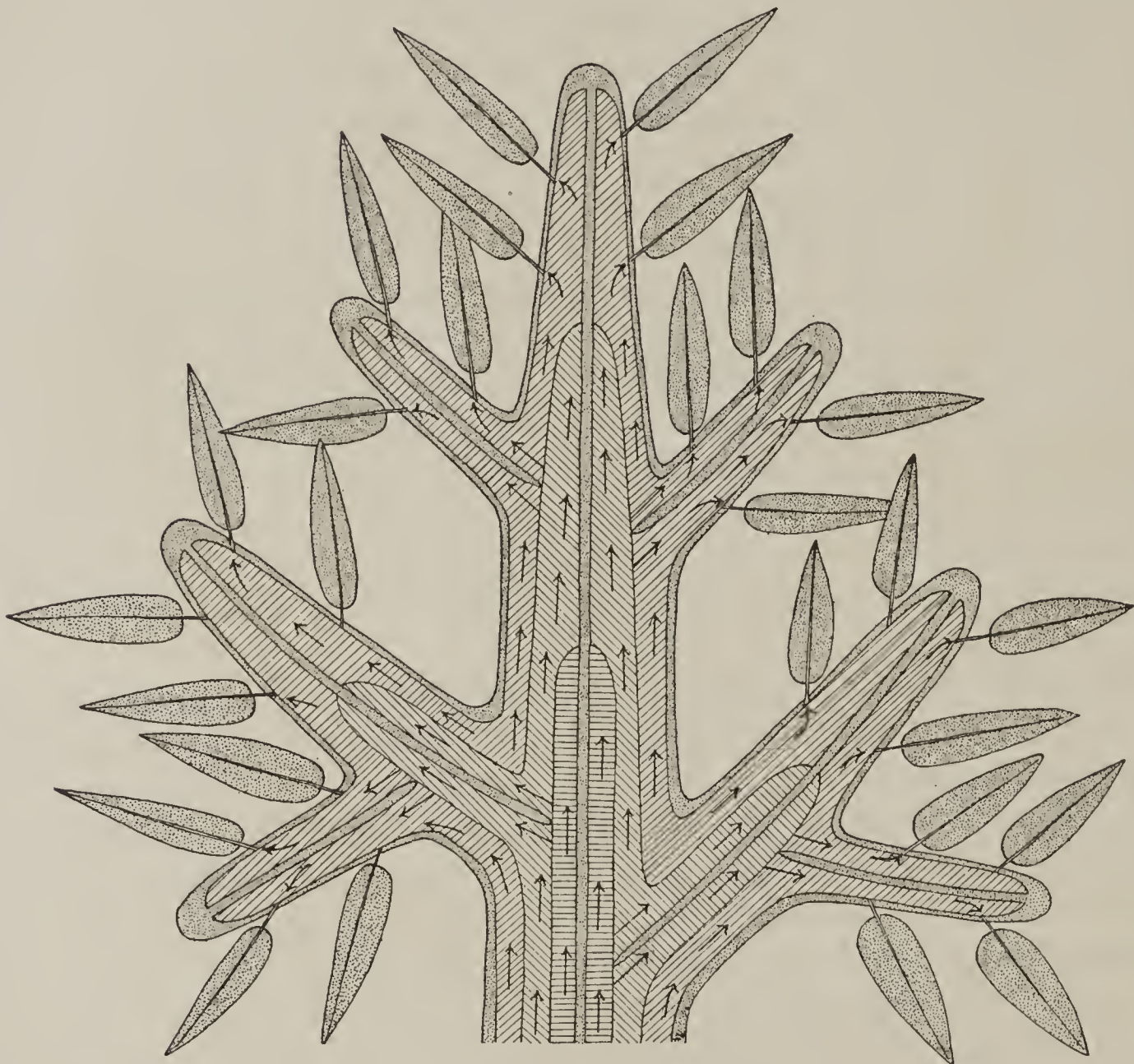


FIG. 19—Diagrammatic longitudinal section of a three-year-old shoot showing, by means of arrows, the path of the transpiration stream when water is entering through all layers. Wood formed the first year is indicated by horizontal shading, that formed the second year by diagonal shading in one direction, and that formed the current year by diagonal shading in the opposite direction.

columns in the xylem of the first year may, therefore, be transmitted to those columns in the xylem of the second year, and thence to those in the xylem of the third, and so on to the columns, when present, in all annual layers.

Although conduction occurs in all portions of the secondary xylem at the apex of each year's growth in a three-year-old stem, there is zonation or limitation of conduction in the basal portions of such three-year-old stems in both the willow and the walnut. Conduction is confined to the outer portions of the summer wood in the willow,

and to the late summer and early spring wood in the walnut. As already explained, page 46, this zonation of conduction is due to gas in specific portions of each annual layer.

Longitudinal extension and lateral thickening of the stem is usually accompanied by the development of lateral branches. Primordia of branches are formed by one-year-old portions of the stem, but do not



FIG. 20—Diagrammatic longitudinal section of a seven-year-old willow shoot (whose leaves are not shown in the figure) showing the upward path of acid fuchsin injected into the four innermost annual layers, but sealed off from the three outermost. Portions of the xylem traversed by the dye are shown in black; portions of annual layers to which the dye had access, but through which it did not travel, are diagonally shaded. Xylem layers from which the dye is sealed off at the bore-hole are shown in white.

usually elongate into branches until the year following their formation. Hence the primary xylem of branches does not abut on the primary xylem of the stem, but upon the outer face of its first year's secondary xylem. Secondary xylem formed by a one-year-old branch is continuous with secondary xylem of the second year in the main axis, and not with the secondary xylem of its first year. It is, therefore, possible that the transpirational pull of a one-year-old branch

upon the stem may be upon the outer face of the first year's xylem, as well as upon the secondary xylem of the current year with which it is directly continuous.

In branches that remain alive for more than one year there is a vertical elongation and formation of new leaves at the apex of the branch, a formation of additional layers of secondary xylem which cap and overlay that of the previous year; a repetition of the process



FIG. 21—Diagrammatic longitudinal section of a six-year-old willow stem which has been stepped in acid fuchsin. The portions of the xylem through which the acid fuchsin travels are shown in black; portions of the xylem through which the dye does not travel are in white.

as described for the main stem. As pointed out, the cylinders of secondary xylem developed by a stem and branch in any given year are continuous with each other, and remain connected after they have been overlaid by successive cylinders of secondary xylem. Therefore, the transpirational pull exerted on the water in any annual ring of a branch or on any portion of it is transmitted only through the corresponding annual layer of the stem. The movement of water and the transmission of forces concerned in its movement through the first,

second, and third year's xylem, and through the succeeding annual layers of xylem, is precisely as described for the tip of the stem, the movement of water and the transpirational forces operating in the same way. (Fig. 19.)

Experiments, in which dyes are introduced into particular annual layers of xylem of the stem, show that the dye passes upward only in these layers and out into the branches only in the corresponding layers which are connected with those in the stem into which the dye was introduced. (Fig. 20.) Such a path is quite different from that obtaining when dyes are entering all annual layers. (Fig. 21.) When the dye reaches the tip of the branch or the tip of the stem, it may pass backward in some of the annual layers, probably on account of a negative force in the cohesive water-columns in the vessels. Apparently the positive transpirational forces which bring the cohesive water-columns into a state of tension may operate in a reverse direction, or downward, and carry the dye downward in other layers after it has reached the tip of the branch or stem.

Of course there is tangential movement in any annual ring so that the dye can move about the buried branch base in the ring in which it is introduced. Therefore, conduction continues in the stem in the layer into which the dye was introduced, as well as passing out into the branch. In some cases where branch bases are deeply buried, and a dye is introduced into a particular annual layer, not only will the dye pass out into the branch in the layer into which it was introduced, but also upward in other layers above the buried branch base. This may perhaps be accounted for by there being a connection between the vessels of the stem and the vessels of the branch in corresponding layers, the vessels of a particular layer being in contact with those of the xylem layers of the stem and branch.

GASES IN TREE-TRUNKS AND THEIR COMMUNICATION WITH THE ATMOSPHERE

Thus far in this paper the following points have been brought out concerning the gases within trees. Gases are present in specific portions of annual layers of several species under consideration, and the presence of gases within the vessels and tracheids results in a definite distribution of the transpiration stream; a distribution demonstrable by the concentric zonation of the colored portions of the xylem in cut ends of trunks stepped in dyes. The various conducting elements containing gases are in vertical communication with one another, and suction or pressures are readily transmitted up or down the trunk to many times the length of the element containing the gases.

Nothing has, however, been said concerning the composition of the internal gases, nor the communication between them and the atmosphere. During the past four years one of us,¹ recognizing the importance of the gas-body within the trunks of trees, has followed the seasonal variations in the proportions of the enclosed gases. Gases liberated in the interior of perennial plants, such as trees, may escape only by solution in the transpiration stream, and by slow diffusion through the cambium and bark. Consequently, air extracted from a tree-trunk has a composition widely different from that of the atmosphere. The proportion of oxygen is invariably less, and that of carbon dioxide greater, than that in the air, and the sum of the two is less than in the atmosphere. Thus, gases withdrawn from a poplar stem (*Populus MacDougalii*) may contain 60 times as much carbon dioxide as the outside air, and this gas was found to constitute 26 per cent of the total amount drawn from oak (*Quercus agrifolia*) trunks in October, 1927, or nearly 90 times as much as in the atmosphere. These conditions could arise only when the production of gas was at a very high rate, and the rate of diffusion was relatively slow, as it would be through the cambium and thence into intercellular spaces communicating with the external air through lenticels or stomata.

The seasonal variation in the composition of the gases is well illustrated by analyses of gases extracted from trunks of *Quercus agrifolia* on 35 dates, beginning October, 1925, and extending to November, 1927. These analyses show a varying carbon dioxide content as shown on page 67.

The season of active growth extends from April to August, and in 1926 an awakening in the autumn was noted which gave high pro-

¹ MacDougal, D. T. The hydrostatic system of trees. Carnegie Inst. Wash. Pub. No. 373, p. 92. 1926.

———. Composition of gases in tree-trunks. Carnegie Inst. Wash. Year Book, No. 25, 160-162. 1925-26.

———. Composition of gases in trunks of trees. Carnegie Inst. Wash. Year Book, No. 26, 162-163. 1926-27.

portions of carbon dioxide in October. Rise of carbon dioxide was seen by May, 1927, which continued until August, a decrease being noted in September, and a still further decrease in October.

1925		1927	
October.....	3.04 to 3.43 per cent	Jan. 17.....	1.4 per cent
		Apr. 15.....	1.5 do.
1926		May 26.....	5.2 do.
June 29.....	9.4 do.	June 23.....	11.4 do.
Aug. 29.....	6.2 do.	Aug. 3.....	15.1 do.
Oct. 16.....	9.4 do.	Sept. 3.....	8.1 to 8.6 do.
Nov. 1.....	3.6 do.	Oct. 22.....	4.7 do.

Salix lasiolepis showed 13.1 per cent carbon dioxide in June, 1926, 8.4 per cent in August, 12 per cent in October, 9.7 per cent in November, 5.2 per cent in January, 1927, 5.0 per cent in May, 8.8 to 9.8 per cent in June, 10 per cent in August, and 10.6 to 10.8 per cent in September. The growing-season of this tree begins in May, with an intermittent period in August and a minor period in October.

Populus MacDougalii at Tucson, Arizona, begins growth about March 1, and continues to enlarge until October, at a rate largely determined by the water-supply. The carbon dioxide content of extracted gas was 1.4 to 2.4 per cent in mid-February, 1927, 6 per cent March 1, 9.8 per cent at the end of March, and on May 8 (at the time of maximum enlargement) 18.2 per cent.

Pinus radiata begins to grow in January and continues to enlarge until July at a rate largely determined by the supply of soil-moisture and temperature of the air. The carbon dioxide content of gases extracted from the trunks of small trees was 3.5 to 4.2 per cent in October, 1925, 12.6 per cent in June, 1926, 4 per cent in August, 5.3 per cent in November, 3.7 per cent in January, 1927, 9.1 per cent in May, 11.2 per cent in June, 11 per cent in August, and 12 per cent about October 1.

The total volume of gas in a tree may be very great. No volumetric data were obtained, but it is probable that the trunk of a willow or of an oak may contain a volume of gas equivalent, at atmospheric pressure, to about one-fourth the total volume of the trunk. A higher proportion is to be expected in some trees. The proportion in any tree would be least in the resting-season when the trunk is said to fill up with water; which may be shortly after the close of the growing-season or immediately before the beginning of the season's activities.

As far as the origin of the gas-body within the trunk is concerned, it is obvious that the source of the carbon dioxide lies in the respiratory processes in the living cells of the rays, the wood parenchyma, and the cambium and its derivatives on the periphery of the woody cylinder. The statement is also widely quoted that in the "ripening"

of wood much carbon dioxide is freed, but we are not able to confirm this conclusion and have made no observations which would bear upon it, except in so far as the seasonal series of gas analyses may furnish data of importance in this connection. Doubtless most of the oxygen and all of the nitrogen present in the gas-body of tree-trunks is to be attributed to their diffusion through the bark, and to the fact that these gases enter the plant dissolved in the soil-water, and may be freed under diminished pressure. The rate of respiration, the varying volume of active cells, the changing solutions of gases entering the roots, possible liberation of gases from ripening wood, separation of leaves and the loss of layers through which gases may pass readily by filtration pressure, dissolving power of sap and the rate of flow of the transpiration stream in which internally freed gases may be dissolved are all factors which may modify the composition of the gases included in vessels and wood-cells. Naturally the integration of the complex effects causes changes which follow the seasons.

The pressure under which the body of gas in a tree exists is subject to wide variations. In dicotyledonous trees, which shed their leaves at the end of the season, the accumulation of water may be so great as to compress the included gases until positive pressures of more than one atmosphere may be found. Such a condition is not likely to occur in evergreen trees, including those conifers which do not shed their leaves at the end of the season. In fact, the gases of most trees are generally subatmospheric as to pressure, as is shown by manometers attached to air-filled bores. Experiments to be described in the next section make it plain, in so far as the trees examined may be depended upon for generalizations, that the pressure of the gas-body in trunks is not usually short of that of atmospheric pressure by more than 0.02 or 0.03 atmosphere. A suction as great as 0.1 atmosphere is rarely demonstrated by air-filled manometers in the oak. When water-filled manometers show greater suction or "negative pressure," it is partly due to the capillary filling of the vessels and other wood-cells opening on to the bores, and partly to the fact that the liquid in the bore is continuous with that of the transpiration stream.

The high percentage of carbon dioxide present in the internal gases of the tree as compared with that found in the atmosphere shows that there is not much communication between the internal gaseous system and the atmosphere. Although there is not a free communication, there are two possible routes by which gases in the interior of the tree may leave or enter the plant. One route is by lateral movement through the trunk; the other is the possibility of escape at the ends of the branches.

The anatomical organization within the stem which seems to permit of the first of the two foregoing possibilities is the system of

intercellular spaces that run radially through the medullary rays. Although most writers agree that there are these radial systems of intercellular spaces in mature portions of the ray, it is not at all clear that they hold that the spaces extend radially through the cambium and its immediate derivatives. The statement that "the cambium cells (of gymnosperms and dicotyledons) fit together without intercellular spaces and form radial rows"¹ may be taken to represent the prevailing conception as to the mechanical arrangement of the cambial cells and their immediate derivatives. That these intercellular spaces in mature portions of the medullary rays also extend through the ray-initials of the cambium is a fact which has been pointed out to us by Professor I. W. Bailey in a verbal communication. This morphologically possible means of communication between the interior and the periphery of the stem is in harmony with facts to be presented on following pages. A second layer which may prevent the radial movement of gases is the phellogen, but it is not at all certain that this layer is without intercellular spaces, especially in portions that lie immediately beneath the lenticels.

Experiments described elsewhere (page 41) show that there is a free vertical transmission of suction and pressures within the trunks of trees. Another series of experiments was arranged to determine whether or not gases could be made to enter the trunk radially by lowering the gaseous pressure within the trunk. Such radial entrance would involve the passage of the gas through an intact layer of living cells, the cambium. In November, 1927, a small oak tree 10 cm. in diameter at the base and 5 meters in length, including the branches, was selected for experimentation. The total volume of the xylem was estimated to be about 25,000 c. c., but, since the vessels and tracheids do not comprise more than one-third of the total volume of the xylem (see fig. 7, page 27), it was assumed that the total gas volume within the tree could not be more than 8 liters, even if all the vessels and tracheids were filled with gas.

A vacuum-chamber of 20 liters capacity and connected with a vacuum-pump was attached to a bore-hole 20 cm. from the base of the tree. Bore-holes 0.5 and 1.0 meter above this were connected with vertical glass tubes standing in dishes of mercury. When the vacuum-chamber was connected and allowed to remain so, there was an almost immediate response in the barometric columns in the tubes attached to the other two bores. Suction rose most rapidly in the lowermost bore, but after 6 minutes the column connected with the bore 0.5 meter from the suction stood at 325 mm. Hg., and the other at 300 mm. The further changes in the barometric columns connected with the various bores are shown in tabular form on the following page.

¹ Strasburger's textbook of botany. Fifth English Edition, 1921, p. 144.

Time	Elapsed time	Suction in mm. of Hg.			Notes
		At bore	At 50-cm. level	At 1-m. level	
7 ^b 40 ^m a. m...	0 m.....	740	0	0	{The rising sun now shone directly on the vacuum-chamber, increasing its temperature and decreasing its suction
7 46 a. m...	6 m.....	325	300	
7 55 a. m...	15 m.....	355	310	
8 05 a. m...	25 m.....	355	318	
8 25 a. m...	45 m.....	355	310	
8 40 a. m...	1 h.....	345	305	
9 40 a. m...	2 h.....	335	295	
10 50 a. m...	3 h. 10 m....	320	280	
4 00 p. m...	8 h. 20 m....	270	270	
	48 h.....	110	110	90	
					{Vacuum-chamber now shaded from sun

The foregoing table shows that following the rise of suction, as indicated by the barometric columns connected with the two upper bore-holes, there is a gradual decrease until the two stand at approximately the same partial pressure as that of the vacuum-chamber. The change from a suction of 740 mm. Hg. to 110 mm. in a vacuum-chamber of 20 liters capacity would necessitate the entrance of about 12 liters of air into the vacuum-chamber. Since 12 liters is considerably more than the estimated volume of 8 liters of gas contained within the vessels and tracheids, it follows that the suction from the vacuum-chamber has pulled air into the trunk from the exterior. Thus, allowing for air pulled in through the barometric columns as set up it may be said that in 48 hours an additional volume of gas amounting to 50 per cent of that already in the trunk was pulled into and out of the trunk. The only alternative would be to suppose that gas had been set free from solution in the sap by the lessened pressure, an action that could not reasonably be held responsible for the great volume of gas concerned.

There is the possibility that in the above-described experiment the amount of gas obtained greater than that originally within the tree might be the result of an intake of gas through the ends of the branches or through the leaves. Accordingly, the trunk of the tree was cut at a height of 172 cm. from the base, the remaining branch removed and the cut surfaces sealed with heavy grease. The barometric tubes were inspected and the vacuum-chamber which had been exhausted to hold up a column of 740 mm. Hg. again connected to the lower bore. The barometric column connected with the 0.5-meter bore showed a suction of 457 mm. in 6 minutes, and that at the 1-meter level a rise to 315 mm., where it became stationary. At the end of 15 minutes after the vacuum-chamber was connected both columns had fallen 5 mm. The chamber was left in connection over night, and both columns were down to 15 mm. on the following

morning. The chamber was again evacuated and connected with the bore to test the fittings. The columns were pulled up as before, but fell quickly. Since the results obtained in this experiment duplicate in a general way those of the previous experiment, it is obvious that the terminal portion of the tree is not, in this case, the chief factor affecting the amount of gas entering the stem, but such gas as enters the stem from the outside comes in radially.

The experiments just described seem to show that gases of the atmosphere may enter tree-trunks radially. Simple experiments described in most textbooks of plant physiology, demonstrate the passage of air through stomata, spongy parenchyma, and petiole of the leaf, and through the lenticels in the cortex of young branches. A similar set of experiments was performed by taking a branch of an oak (*Quercus agrifolia*), 40 cm. in length and 6 cm. in diameter at the base, and with three leafy shoots—one 40 cm., one 35 cm., and another 32 cm. in length—and sealing it into a flask, attached to a vacuum-chamber, with the lower cut end of the branch in water. All portions of the branch and branchlets, except the leaves, were carefully covered with shellac to seal all lenticels and other rifts in the bark. The terminal and lateral buds were also sealed with shellac. On application of a suction of 740 mm. Hg., air was drawn in through the leaves and streamed out of the cut end of the xylem under water in profuse quantities. Care was taken to allow the experiment to proceed long enough to be certain that all gas was exhausted from the wood of the branch, before concluding that the air coming through the apparatus was that taken in from the atmosphere through the leaves. To show that this air came in through the leaves, all the leaves were covered with a coating of hard grease so as to securely seal all wounds and stomata. Suction was again applied with the result that no air was drawn into the plant, as was evidenced by the absence of bubbles from the cut end in water. The remaining greased branch was severed and its cut end placed under water in the flask attached to the vacuum-chamber. Suction was again applied with the same results as above. No air was drawn in through the sealed leaves and stem at a suction of 740 mm. Hg.

Further experiments with branches of this oak showed that there could be an entrance of air through the lenticels, and a lateral movement of the entering air through the cambium to and downward through the xylem. This was demonstrated by taking a branch 25 cm. in length and stripping off the bark from the lower end immersed in the water of the flask, connected with the vacuum-chamber, and securely sealing with hard grease the upper cut end exposed to the air. When suction was applied to this branch there was a steady stream of bubbles from the end of the branch immersed in water. A similar experiment in which the portion of the branch immersed in

the water had an intact bark showed bubbles streaming from both the cut end of the xylem and the lenticels. This is taken to show that air entering through the lenticels in the upper part of the branch may either move into the xylem and thence downward and out through the cut end of the xylem, or move directly downward through the inner portion of the bark and thence out through the lenticels in the lower part of the bark. Further demonstration of the movement of gases through the inner part of the bark only was given by an experiment in which both cut ends were securely sealed with heavy grease. In this case the bubbles streaming out of the lower part of the branch came from the lenticels only. There is the possibility that in the foregoing experiment, in which the cut ends of the xylem were sealed off, the air moved from lenticels to xylem, downward through the xylem, and thence out through the lenticels. To show that this is not the case, a strip of bark was placed in the suction apparatus with all portions of the bark, except the surface exposed to the air in intact branches, sealed off with heavy grease. When suction was applied to the strips of bark treated in this fashion, there was the same escape of bubbles from the lower lenticels as when intact ends of branches were immersed in water in a vessel connected to a vacuum-chamber.

At first glance, this demonstration of free movement of gases between the interior and exterior of terminal portions of the oak (from leaves to the xylem of the branch, radially through the lenticels, or vertically through the inner bark) seems to be out of harmony with the fact that old trunks with intact branches, and trunks with the branch system removed, behave the same with respect to suction applied to the internal gaseous systems (page 69). One would expect that suction of 0.5 to 1.0 atmosphere applied at the base of the trunk would draw air in to the trunk through the terminal portions. The reason why there is not this equalization of pressure under these conditions is probably due to the great distance to be traveled and the resistance the gases encounter in passing from one vessel to another; a gradient in resistance to passage which is evident in manometers within a meter from each other (page 41). This resistance to equalization also increases with the volume of the trunk, as is evidenced by the behavior of the gas-body within large and small trees.

Since *Quercus agrifolia* always retains some of its leaves, some further experiments on the permeability of terminal shoots to air were made on *Juglans major*, a tree which sheds its leaves. A shoot bearing two branches, which had shed their leaves about a month previously, was tested to ascertain whether or not air could be made to pass into the vessels under suction. The basal portion of the shoot, stripped of its bark for a distance of 8 cm., was sealed into a flask with the end under water. The flask was connected with a vacuum-cham-

ber. A suction of 740 mm. Hg. was applied long enough (4 p. m. November 7 to 8 a. m. November 8) to be certain all air was exhausted from the stem and branches. Even after 48 hours a continuous stream of bubbles was observed issuing from the cut end under water and also from the bared surface of the wood. From this experiment it may be concluded that branches which have lost their leaves are also freely permeable to the gases of the atmosphere when the pressure within the plant is less than that of the atmosphere.

Some further conception as to the size or character of the openings through which gases enter may be obtained by thrusting leafless branches of *Juglans* into a solution of acid fuchsin, or of a more soluble dye, such as Bismarck brown, and applying a suction of 740 mm. Hg. to the base of the branch. When suction is so applied there is no visible disappearance of the dye, and dissection of the branch shows that there is no coloration of the internal tissues. This may be taken to show that watery solutions may not be drawn into living uninjured stems by suction which cause a free entrance of gases. It is very probable, therefore, that the openings through the bark and cambium are not of such magnitude or character as to allow the passage of solutions under filtration-pressures, but will allow the passage of gases under similar pressures.

It should be pointed out that the sub-atmospheric pressures of 0.5 to 1.0 atmosphere used in the above experimentation seldom if ever obtain in intact trees. Because there are not these marked differences in pressure between the exterior and interior of trees under natural conditions, gaseous interchange between the inside and the outside of the tree probably is so slow that concentrations of carbon dioxide greater than, and the concentrations of oxygen less than, that of the atmosphere may be found within the trunk.

The trunks of trees are to be visualized as woody cylinders, the older layers, or parts of each annual layer, of which contain oxygen, carbon dioxide, and nitrogen in proportions widely different from those in which they occur in the atmosphere. These differences imply that the channels of exchange have a very low capacity for diffusional movement of such gases. The application of pressures no greater than 0.1 atmosphere will, however, be followed by passage of air in notable volume. The dimensions or character of the passages are such that watery solutions may not be forced through them by a pressure less than 1 atmosphere.

Two important implications arise from these conditions. One is to the effect that the actual "negative pressure" of the gases inside the tree is rarely as much as half an atmosphere. No such amount has been encountered in any of our experiments with air-filled manometers. However, when bores filled with water are connected with manometers, suction greater than the above amount may be regis-

tered, but these effects include capillary filling with water of vessels and other elements opening on to the bores, and other abnormal conditions. It may be safely assumed that any reduction of the gas-pressure in the wood of a tree to a point approaching half an atmosphere will be followed by the entrance of gases through the bark and along the intercellular spaces of the rays. On the other hand, after the close of the growing-season, when the trunk "fills" with water, a positive pressure over 1.1 atmospheres greater than that of the air may be set up. A possible explanation is that, under such conditions, the intercellular passages may become injected with water, a filling which would prevent an escape of internal gases sufficient to equalize the internal and external partial pressures.

It is conceivable, also, that the rise in carbon dioxide content of internal gases, which follows shortly after rainstorms, may be due in part to a similar sealing action of water entering the passages from without.

There should also be mentioned, in this connection, the relation of the tensions in the hydrostatic and pneumatic systems. The gases in vessels and tracheids may move longitudinally by passing perforations in the closing membranes of bordered pits between adjacent elements of the same kind, as well as radially through intercellular spaces in the rays and ray-initials of the cambium. The ready longitudinal communication of variations in pressure, which have been described elsewhere, may possibly result in a condition in which the pressure in the pneumatic system throughout the trunk and larger branches presents only minor and temporary differences. Under such conditions equalization between changes in pressure in the upper and lower part of even a tall trunk would take place within a period of a few minutes or a few hours.

Much confusion has resulted from the widely prevalent failure to differentiate tensions in the pneumatic and hydrostatic systems. Variations in the pressure of gases in the wood can have only a negligible effect on the meshwork of ascending sap. At the same time the tension in the water system does not exert a direct suction on the pneumatic system. The pneumatic system communicates with the hydrostatic system only through perforations in the closing membranes of pits, and through the still more minute openings in walls, such as the submicroscopic cavities in the water-filled walls abutting on the lumina of elements filled with gas. These spaces leading out from conducting elements are filled with water. It is only when the tension in the hydrostatic system reaches a point where the water is pulled from these communicating tubelets that gas is drawn into a conducting element, thereby eliminating it from the conducting system. The actual suction on the gaseous system is simply that which results from the diminution in the volume of water in the conducting

tracts by reason of increased tension brought about by transpiration. A pull of 100 atmospheres originating in the transpiring cells of the leaves may exert enough force (or suction) to withdraw water from the minute passages in the walls of certain conducting tracts abutting on the gas-filled elements, and thus allow gas to pass into the vacated cavities, and thence into the conducting elements in which the entering gas may collect as a bubble.

The conditions could be illustrated by an arrangement in which a tall pipe of cement or clay filled with water, and with its lower end in water, is provided with some device for exerting a pull of a hundred atmospheres on the upper end of a water-column. If the water "wets" the walls or occupies spaces near the lower limit of size which may be occupied by water, the frictional resistance to movement in these spaces will be so great that no suction whatever will be exerted on the surrounding air. If, however, the upward pull did reach a magnitude in which water was extracted from a wall-space, air would be pulled into the space, and the process would continue until air filled the space and collected as a giant bubble: this would eventually put the giant cell out of commission as a water conduit.

SUCTION AND PRESSURE

A recognition of the mechanical diversity of the arrangement of the conducting systems in different genera of trees detracts much from the values of results obtained by comparisons of observations made with water-filled manometers attached to stumps of stems or branches, or connected with bore-holes in trunks. As has been intimated in a previous publication,¹ the great mass of records of observations of pressures in stems, roots, and branches can not be safely used for comparisons, since but little information is given as to the tissues penetrated by the bores; while in manometers attached to stumps the results are so complex as to be difficult to interpret.

The senior author began a series of manometric observations on *Quercus*, *Pinus*, *Juglans*, *Salix*, and *Sequoia* in 1923, which have been continued to the present time. A portion of the observations has been published in the paper cited in the previous paragraph. Manometers were kept on the cut ends of a pine root since July, 1925, with renewal of the fitting twice annually (fig. 5, Pub. 373) until late in 1927. Similar observations have been made for a longer time with manometers attached to the terminal stump of the stem of a small pine tree (fig. 6, Pub. 373). Other manometric series have been followed for periods of a year or more.

An analysis of the records, which are held to be much more extensive than those of any series previously made by any one, shows that the readings of a manometric gage connected by a water-column with a bore-hole or stump do not indicate the static condition of the water-column or of the gas-body. When a bore penetrating the regions occupied by the cohesive mesh of sap, and also inevitably some gas-filled elements, is made and filled with water, capillary action is set up; water enters some of the elements occupied by gases, a portion is dissolved, some is forced out and comes into the manometer from which it must be released by the operator. The connection of the water in the bore with the cohesive mesh subjects it to the pull from the leaves. If the water in the bore was thus connected strictly with the ascending transpiration stream, the manometer should register "negative pressure" as the total force of the pull. The water in the bore, however, also runs into the capillary elements extending into the gas-filled part of the wood, which constitute an elastic cushion so that the full force of the pull is never shown by water-filled manometers. A commonplace comparison would be afforded by the results of sucking lemonade up through a straw or a glass tube, and through a lamp-wick. The pull would be registered directly and simply in the first case, but would not be in the second.

¹ MacDougal, D. T. The hydrostatic system of trees. Carnegie Inst. Wash. Pub. No. 373. 1926.

It is evident that the practical impossibility of making two or more bores identical as to connection with the water-column, or the gas-filled vessels or tracheids in a trunk, is in large degree accountable for the lack of concordance of results of manometers arranged in series up and down the length of a tree. The fact that greater suction is not uniformly shown by manometers near the summit of a tree has been adduced as an argument against the cohesion theory of the ascent of sap. The implication is not justifiable in the light of the conditions described.

A manometer attached to a bore-hole in a pine tree will show exudation-pressures as high as 4 to 5 atmospheres set up by the hydration and expansion of the contents of the resin-canals which were tapped. Similar positive pressures may be observed in stems with a latex system, and in the massive cortex of *Carnegia* where great masses of thin-walled cells are hydrated and expanded as a result of the treatment. These pressures are not manifest in the undisturbed cell-masses. Similar action has been widely observed on stumps of palms, grape-vines, and many other plants.

There are, however, a number of manometric observations of positive pressures in roots which may not be so simply explained, and of which no interpretation is available at the present time.

Suction or "negative pressure" has been widely observed by the use of water-filled manometers attached to bores and stumps of roots and shrubs. A manometer connected with a water-filled bore-hole penetrating the outer layers of a trunk in which the cohesive column of water is present, and in which an upward movement is taking place most rapidly, will show suction. Some of the suction is due to the capillary advance of liquid in all directions from the bore-hole. In no case may the suction be taken to be a measure of the transpiratory pull, though it is seen to increase with heightened water-loss and to diminish with lessened transpiration.

The best opportunities for making definite connections with regions chiefly occupied by the cohesive mesh of water in which upward movement takes place, and of putting other instruments to register pressures of the regions internal to the water-column, are to be found in pine trees which make heavy annual layers. The total thickness of wood formed during the previous three or four seasons, and through which the transpiration stream moves most rapidly, may amount to as much as 5 cm. It is possible to drive a bore tangentially into these layers so that a minimum of air-filled tracheids are cut into. After the first exudation of resin causing positive pressures has passed, and the manometers are refitted to the cleaned bores, suction is registered the variations in which run parallel to the changes in the tension of the cohesive column. Suction rises from early in the morning

until after midday, and then decreases as transpiration slows down, as is amply illustrated by data given in Pub. 373.

If a second bore no more than a few centimeters distant from such a tangential bore be driven radially into a small pine tree (see fig. 22) with the metal tap screwed in as far as possible to shut off layers through which conduction is most rapid, connection will be made with the older central layers of tracheids which contain air and which conduct more slowly. Manometers connected with such bores show variations widely different from those attached tangentially, showing least suction or even pressure in the midday period when suction is greatest in the tangential layers. Part of this action may be ascribed to the expansion of the gases by temperature.



FIG. 22—Transverse section of a trunk of an eight-year-old Monterey pine, cut down in 1926, showing, at the level of the section, the tangential bore which tapped the three outer annual layers, and, at a slightly lower level, the radial bore which tapped all annual layers.

The greater part of available information concerning suction and pressure in shoots has been derived from observations with small stems and branches of woody or herbaceous plants. When a branch or a leafy shoot is detached, the entire water- and gas-system is cut across. If this is done in the air, the sub-atmospheric pressure in the gases of the vessels and tracheids, and the tension in the water-mesh immediately sets up abnormal conditions. The pressure in the vessels is equalized, and the water in the conducting tracts in the basal part of the detached stem or branch is pulled upward, allowing air to enter the conducting tracts and to break the strands of water, so that wilt-

ing generally results before the continuity of the water-columns can be restored.

The possibility of connecting manometers with bores which might be arranged to gain access to the gas of the interior with a minimum of disturbance of, or connection with, the transpiration stream, does not seem to have been seriously considered in experiments outside of those made in connection with the researches in this laboratory. At any rate, but little reliable information is available as to observations made with manometers in which a column of air or of gases is interposed between the mercury-column and the surfaces of the bore-holes. Nearly all practice has been based on the erroneous assumption that all cut surfaces should be wetted as soon as possible after being bared.

A bore driven into a tree cuts through strands of water in tracheids or vessels, but the number thus disturbed is so small in comparison with the number of undisturbed elements that the ascent of sap is not altered in any measurable manner. The part of the bore which cuts air-filled elements allows direct communication through the vessels for long distances up and down the tree, so that differences in pressure may be transmitted within a few seconds to a distance of 3 or 4 meters in oak and willow trunks (see page 42). The actual rate of movement of gases in the trunks is denoted by the fact that the gas-extraction apparatus with the receiver of mercury set at a level to give a suction of 0.4 to 0.6 atmosphere pulls 200 c. c. of gas at atmospheric pressure from a 12-cm. bore-hole in 4 to 6 hours.

It follows that a manometer sealed into an air-filled bore will show the pressure in the gases in the trunk quite correctly. Large vessels, like those of the oak, remain open without clogging for many days. Elements which contained water when cut across do not affect the transmission of gaseous pressures or suctions through air-filled vessels, and the actual disturbance to the entire system is very slight. If an experiment of this kind were made at the time when the trunk is filling up with water, some of the liquid would appear in the bore and might thus be transferred more readily to other elements than in intact wood. The results at this time would be quite like those coming from spraying or filling a bore with water.

A series of observations to obtain data for an analysis of the conditions of the air-body within trunks of the oak (*Quercus agrifolia*) were made in the summer of 1927. A water-filled manometer had been in operation on a tree about 25 to 30 cm. in diameter for three seasons, during which time it had been refitted to two new bore-holes each season. During the period from June to October it was still attached to a bore to which it had been fitted in May. A manometer consisting of a vertical tube standing in a dish of mercury was connected with a metal screw-tap inserted in a 12-cm. bore-hole fitted to a tree of similar size standing 2 meters distant. No water was

included. A third instrument was attached in like manner, but was provided with a T-tube connection so that the air in the manometer, about 50 c.c., could be evacuated at will by a vacuum-pump, the mercury column in a barometric gage attached being pulled up to 740 mm.

The three manometers were set in action June 23 and operated for a week. The water-manometer during this time showed registrations of suctions of 186–192 mm. Hg. The manometer connected to the pump was evacuated on alternate days until the gage showed a suction of about 740 mm. The suction fell to 212–80 mm. Hg. within 24 hours, and to 50 mm. in the third day; the single occasion when the experiment was allowed to run undisturbed for that period. The air-manometer, which was not manipulated, showed suctions of 10 to 12 mm. Hg. during the week.

The manometer with pump attachment was now moved to another tree slightly smaller, and a carboy with a capacity of 20 liters was connected in the system. Observations with the tree instruments were begun on July 7. The carboy was evacuated until the barometric gage showed a suction of 750 mm. Hg. Gas was slowly pulled out of the tree, so that the suction fell to 600 mm. on the 8th, 390 mm. on the 10th, 240 on the 12th, 150 on the 14th, 110 on the 28th, and 107 mm. on August 4, at which time the pump was connected and the carboy again exhausted until the barometric gage again showed a suction of 740 mm. Hg. During this period of over a month the air-manometer showed suction of 3 to 12 mm. Hg.

The carboy was now left in connection with the bore-hole. The connecting stopcocks were turned at infrequent intervals to test conditions of pressure. The suction varied from 95 to 110 mm. Hg. A reading of 75 mm. on October 23, at 10 a. m., and of 90 mm. at 8 a. m. of the following day was made, showing that the entire air system of the tree was below atmospheric pressure. The water-manometer gave readings not widely different. This day and the previous week had been cool, with very little sunshine; conditions under which air- and water-filled manometers might be expected to approximate most closely.

The water-manometer was emptied of the liquid on October 28, and readings were taken with a cushion of air in the bore. On October 29, a day with an overcast sky and relative humidity near the saturation point, there was a suction of 16 mm. Hg.

Gas samples had been taken from another small oak tree, standing within 3 meters of the first, for a week. About 3 liters had been extracted, using a mercury-column with a suction of 0.4 to 0.6 atmosphere. The extraction apparatus was disconnected on the 29th at 10^h 30^m a. m., and a manometer connected with the bore without the introduction of water. The extracted gas samples had been charac-

terized by a high carbon dioxide content, 19 to 26 per cent of the total volume, which was much over that of any gas sample taken from any tree.

Although it was established by experiments described in another section of this paper (pages 41 to 45), that pressures might be quickly conducted to distant parts of the stem, it was found in the above test that the volume of the gas-body within the trunk was so large that an extraction of a few cubic centimeters by suction did not affect the readings of water- and air-manometers attached to bores within a meter. The experimentation was now transferred to a small tree. The larger tree had a volume of about 90 liters of enclosed gases. The smaller tree did not contain more than one-tenth of this quantity.

A bore was made in the small tree, 2 meters from the base, and connected with a pump with a vacuum-chamber of 20 liters capacity. The pump when operated was capable of setting up a suction of about 740 mm. Hg. It was operated intermittently, the suction of the vacuum-chamber being allowed to run down as gas was extracted from the tree-trunk. An air-filled manometer was attached to a bore about 1 meter below the pump connection, and a water-filled manometer to a bore 60 cm. lower on the opposite side of the tree. The tree was about 12 cm. in diameter, and the bores were 5 to 6 cm. in depth, penetrating to the center.

At the beginning of the experiment the pump was operated for a few minutes ending at 9 a. m. August 15, bringing the suction up to 740 mm. The water-manometer had been put in place the previous day, and showed a suction of 80 mm. Hg. At 10^h 40^m a. m. the suction of the vacuum-chamber was down to 720 mm. The air-manometer which had shown no suction at the beginning of the experiment now showed a suction of 8 mm. Hg., and the water-manometer showed a suction of 180 mm. Hg.

At 8 a. m. the following day the vacuum-chamber had extracted so much gas that its gage had fallen to a suction of 320 mm., the air-manometer showed a suction which had increased to 45 mm., while the suction of the water-manometer had fallen to 96 mm. Observations were continued for a week, the changes in the bores being indicated in the table on page 82.

Another series of experiments was made to test the communication of lessened pressures or suctions through the length of an oak tree. A vacuum-chamber of 20 liters capacity was exhausted until it held up a barometric column of 740 mm. Hg. without leakage. This was connected by a stopcock with a tap screwed into a bore 30 cm. from the base of a small oak whose diameter was 12 cm. at the ground level. An air-manometer was affixed to a bore 50 cm. directly above the connection with the vacuum-chamber, a second at 1 meter above,

and a third at the 1.5-meter level. A small branch with a limited leaf-surface arose from the trunk at a point between the first and second manometers, and on the opposite side of the tree. When the vacuum was connected at 8^h 52^m a. m., the suction in the lowermost

TABLE SHOWING THE EFFECTS OF CONTINUED SUCTION FROM A VACUUM-CHAMBER UPON THE GAS-BODY WITHIN THE TRUNK OF AN OAK

Date	Time	Suction in mm. of Hg.			Notes
		Vacuum-chamber	Air-manometer	Water-manometer	
Aug. 16	8 ^h 30 ^m a. m. . . .	750	70	155	
	8 55 a. m. . . .	748	88	175	
	11 00 a. m. . . .	660 ¹	100	170	¹ Suction of chamber re-
	11 40 a. m. . . .	733	100	172	stored to 740 mm.
	1 40 p. m. . . .	645 ²	96	157	² Suction of chamber re-
Aug. 17	7 20 a. m. . . .	370 ³	67	116	stored to 740 mm.
	11 00 a. m. . . .	740 ⁴	102	172	³ Suction of chamber re-
	12 00 noon. . . .	735	102	164	stored to 740 mm.
Aug. 18	8 00 a. m. . . .	38 ⁵	0	15	⁴ Suction restored by pump.
	10 30 a. m. . . .	740	110	172	⁵ Pump now operated con-
	11 40 a. m. . . .	725	110	168	tinuously for 2½ hours.
	2 45 p. m. . . .	720 ⁶	100	145	Rise in water-manometer
	3 45 p. m. . . .	740	108	145	in 5 minutes; no rise in
Aug. 19	4 15 p. m. . . .	740	110	145	air-manometer in 10 min-
	7 30 a. m. . . .	160 ⁷	33	25	utes.
	8 00 a. m. . . .	740	65	140	⁶ Suction of chamber re-
	9 00 a. m. . . .	740	101	162	stored to 740 mm. and
	10 20 a. m. . . .	740	112	156	held so.
Aug. 20	11 25 a. m. . . .	703	115	155	⁷ Chamber maintained at
	7 30 a. m. . . .	45 ⁸	20	0	740 mm. from 7 ^h 30 ^m to
	8 00 a. m. . . .	735	53	113	10 ^h 20 ^m a. m.
	8 30 a. m. . . .	730	98	162	⁸ Suction of chamber re-
Aug. 22	2 40 p. m. . . .	325	136	325	stored to 740 mm.
	7 30 a. m. . . .	35 ⁹	0	6	⁹ Suction of chamber re-
	10 45 a. m. . . .	723	20	158	stored to 740 mm.
	4 30 p. m. . . .	710	48	80	
A new bore for the vacuum-chamber now made 36 cm. lower on trunk that is, 80 cm. from the water-manometer and 60 cm. from the air-manometer. The pump connected with the vacuum-chamber was started at 8 ^h 05 ^m a. m. and operated continuously all day.					
Aug. 23	8 ^h 35 ^m a. m. . . .	746	12	15	¹⁰ Air-manometers on con-
	10 20 a. m. . . .	746	68	85	trol tree registered 8-16
	11 20 a. m. . . .	746	75	110	mm. Hg.
	1 45 p. m. . . .	743	106	145	¹¹ Water-manometer on con-
	5 00 p. m. . . .	743	150 ¹⁰	110 ¹¹	trol registered 75-82 mm.
					Hg.

manometer, 50 cm. from the connection, pulled air past the mercury-column within a few seconds, the second manometer showed a suction of 50 mm. of Hg. within two minutes, and the third showed 25 mm. within four minutes from the time when suction was first applied. All instruments returned to 0 within ten minutes. The manometer 50 cm. from the suction-bore was replaced with a barometric column.

Suction was again applied, and the barometric column 50 cm. from

the connection with the vacuum-chamber showed a suction of 300 mm. Hg.; the manometer at the one-meter level a suction of 100 mm. within 100 seconds. A few bubbles of air were pulled past the U of mercury, and the suction from the vacuum-chamber was cut off. All manometers returned to 0 within ten minutes. A special test of the speed of communication was made by turning the stopcock connecting the vacuum-chamber, now standing at 400 mm. Hg., and noting the time within which the lessened pressure was recorded by the various manometers. The response was so immediate in the manometer at the 50-cm. level that no time can be given: movement of the mercury in the manometer at the meter-and-a-half level took place within 4 seconds. The length of vessels in this oak has been found to be 33 to 35 cm.

Late in the afternoon the vacuum-chamber was again connected by turning the stopcock. The effect on the various instruments was practically instantaneous, and the lessened pressure showed a speed of transmission much like that of the speed of increased pressures described on page 41.

The most striking feature of the above-described observations is the fact that the suctions registered by water-manometers do not correspond with those registered by air-manometers. In water-filled manometers the bore-holes also contain water, from which there is a capillary filling of the vessels and the other wood-cells in the neighborhood of the bore. This capillary filling of the tissues is manifested in the manometers as suction, and coincident with the suction set up by transpiration. Whereas, in the air-filled manometers changes registered are to be ascribed only to changes in the gaseous system internal to the tree. As just stated, the connections of the two types of instruments should by no means be taken to be equivalent. Suction in the water-filled bore would at all times be much greater than that shown by an air-manometer. When suction was applied by the pump the effect was invariably greater in the water-filled bore than in one containing air, although the distance from the pump to the water-cushion was nearly twice that to the air-manometer.

The second series of tests are valuable principally in demonstrating the communication of air through great length of trunk and from one vessel to another. The suction which was exerted for only one or two minutes pulled gases from the vertical sector into which all bores were driven. When the vacuum-chamber was disconnected the partial vacuum set up in the sector of the trunk pulled gases from the remainder of the trunk, so that the pressure was brought back near to the original; a pressure not far from that of the atmosphere as shown by readings before the vacuum-chamber was connected. No tree has ever shown by its own action a suction of more than 16 mm. Hg. on air-filled manometers.

In conclusion, it may be stated that air-manometers attached to tree-trunks show that suction is transmitted to considerable distances along the gas system within the trunk, and that these suction can be maintained for days by operating a vacuum-pump from time to time. The effect of this reduction of the gaseous pressure within the trunk is to increase the suction of the hydrostatic system, and water-filled manometers register suction of 155 to 175 mm. Hg. instead of about 80 mm. In a very broad way, reduction of the internal pressure of the gaseous system is accompanied by an increase in the transpirational pull.

Increase in the internal gaseous pressure within the trunk does not, however, have a corresponding effect upon manometers connected to the hydrostatic system, and there is no essential change in the suction recorded by water-manometers when pressures of 3 to 4 atmospheres are applied through bore-holes to the internal gaseous system. It is clear that varying pressures in the internal gas-body exercise only a minor influence on the tension in the cohesive water system.

FACTORS AFFECTING SUCTIONS AND PRESSURES

Owing to the osmotic action of the epidermal cells of the root, especially of the root-hairs, a considerable amount of water and dissolved substances may enter the cortex of the root and pass into the elements of the stele, whose tissues are rather rigidly confined by the surrounding endodermis. The cells of the endodermis function, to a certain extent, in preventing a backward movement of water and dissolved substances into the cortex so that there may develop within the stele a considerable pressure, the so-called "root-pressure," which may be sufficient to force water and dissolved substances into the conducting elements of the xylem. The presence and cause of such a pressure has attracted much attention. Some investigators¹ hold that these pressures, at least in part, are referable to the action of the endodermal mechanism which controls and maintains the process.

Similar exudation-pressures occur elsewhere in the plant, and may result in a forcing of water and dissolved substances into the vessels from abutting parenchymatous cells. Atkins' views as to the causes of this have already been discussed (page 53). He finds that there is a rapid rise in the concentration of the sap in the vessels of some trees towards the end of the season, which he ascribes to a more or less uniform secretion of sugars into the transpiration stream during the time when reduced transpiration entails a diminution in the rate of the current past the secreting cells, just before leaf-fall. He holds that if the rate of secretion remains approximately constant the concentration of the sap will depend upon the volume of the transpiration stream. The complete cessation of transpiration in winter allows a further concentration, a concentration which reaches its maximum simultaneously with, or just previous to, the expansion of the leaves. These results show that variations in the osmotic concentration of the sap are important contributing factors in the accumulation of water in the wood of trees, which, in turn, markedly affect the gaseous and hydrostatic pressures. We have also discussed the views of Jones and his coworkers (page 53) who consider the excess of water-absorption over water-loss in spring, before the leaves unfold, as being due to the conversion of the stored carbohydrates into sugar with a consequent rise of the osmotic concentration of the sap in the vessels. This makes possible the absorption of large quantities of water, not much of which is transpired. They hold that conditions above and below the ground, as well as internal conditions, result in a relatively high sap-pressure and a consequent filling of the trunk with water. It

¹ Priestley, J. H. The mechanism of root-pressure. *New Phytol.*, 19: 189-200. 1920.
———. Further observations upon the mechanism of root-pressure. *Ibid.*, 21: 41-47. 1922.

Overton, J. B. The mechanism of root-pressure and its relation to sap-flow. *Am. Jour. Bot.*, 8: 369-374. 1921.

may well follow that under these conditions the amount of free gas present in the trunk would be relatively small, but that as soon as transpiration, temperature changes, and other changes modify this sap-pressure, some of the vessels may be emptied of water and the water replaced by gas.

It should also be borne in mind that pressures developing within different parts of the trunk are not of the same magnitude. As we have shown on page 78, tangential bores tapping the outer annual layers of the pine register positive pressures, while radial bores to the inner layers register only suction. This is also true, at least under midsummer conditions, in the willow and the walnut. It would appear, therefore, that water is not forced into these trees from any pressure below, and that pressures registered in the trunk are due to the activity of the living cells of the trunk and to the effects of transpiration.

The relation of sap-pressures found in roots to sap-pressures found in stems, especially as affected by changes in external conditions, such as those of soil-moisture and the amount and rate of transpiration, are illustrated by the following experiments.

On June 5, 1926, a manometer was attached to the stump of a sawn-off root of a large willow tree (*Salix lasiolepis*) growing on the edge of a small, sluggish stream. This root had a woody cylinder 3.5 cm. in diameter after the cortex was removed. Daily records were kept from June 5 to July 22, 1926. On sunny days, which were usually warm, there were 8 days when the manometer registered pressures. These pressures ranged from 0 to 35 mm. Hg. On the 15 other sunny days there were suction of from 0 to 62 mm. Hg. Only suction of 0 to 35 mm. Hg. were registered on the remaining 17 days, which were all cool, and foggy a portion of each day. When this willow tree, which has an adequate water-supply, is actively transpiring, it may manifest a positive pressure, in the manometer attached to the root, or it may show a suction. The root always shows a suction on days when the transpiration is reduced. There is no apparent correlation between the transpiration and the root-pressure as registered by manometers.

The fact that decapitated plants, especially herbaceous ones, may show exudation-pressure when sufficiently saturated with water, has led many to attribute the exudation to a pressure brought about by the roots. Confusion arises from ascribing all exudation-pressures to the osmotic action of the root, because all attempts to apply generalizations based upon the study of small trees and on herbaceous plants to roots of large trees overlook one important factor. In small trees and in herbaceous plants the greater portion of the conducting system in the root is ensheathed by the endodermal mechanism. Whereas, in large trees, as in the root of the willow just under con-

sideration, there is no endodermis surrounding the greater part of the conducting system in the root.

Further light upon the factors affecting exudation-pressures of roots is shown by the following experiments. A small oak (*Quercus agrifolia*) whose trunk was 13 cm. long and 1 cm. in diameter was decapitated 8 cm. above the root system. A manometer was attached to the stump and the soil surrounding the plant profusely watered.¹ The first few days after decapitation the stump showed slight exudation-pressures, after which a very marked suction set in and continued until the end of the experiment. Variations in the suction-pressure were more or less coincident with variations in the temperature, although the gas-body within the stump was very small. The suctions obtained can not be ascribed to the death of the stump, since it put forth several new branches following the removal of the manometer. A small Monterey pine treated in similar manner showed only suction. These two experiments give no basis for a conclusion that osmotic action of the root is sufficient to assist in forcing water up the stem.

That such variations in pressure depend not only on the amount of water in contact with the roots and temperature variations, but also upon the volume of the plant left for experimentation after decapitation, may be shown as follows. Two small walnut trees (*Juglans regia*) of approximately the same size, 1.5 meters in height and 2.5 cm. in diameter, were decapitated on July 28, 1927; one 11 cm. and the other 90 cm. above the soil. Both stumps were profusely irrigated. The short stump at first showed a slight pressure and then a marked suction; the longer stump always showed a marked suction. The bark of each stump was covered with heavy grease to prevent lenticular evaporation. Both plants eventually showed a greater suction than that manifested by similar but defoliated ones, or even by similar plants with their leaves intact. The experiment ran from July 28 to August 31, 1927.

The foregoing experiment seems to show that the manometric variations produced by stumps are not ascribable entirely either to the activity of the living cells of the roots, or to that of cells in the neighborhood of the wound, or to changes in the hydrostatic system caused by transpiration and evaporation from lenticels, or to expansion of the enclosed gas-body. Differences in suctions recorded by the manometers may be largely due to capillary absorption and to hydration of the tissues and their constituents. It is evident that the amount of water in the soil affects the phenomena of swelling and the consequent manometric measurements. Such hydration of plasmic

¹It should be remembered that these experiments have all been performed during June, July, and August, in a region where there is a long rainless summer following the cessation of the winter rains in the latter part of March.

masses and capillary filling of the vessels and of other wood-cells may well be important factors affecting pressures manifested in manometers attached to bore-holes, or to stumps of stems or branches. It would appear that the phenomenon of bleeding, especially evident in the spring before the buds are open, is not to be ascribed solely to the phenomena of root-pressure, sap-pressure, or exudation-pressure (and the resultant filling-up of the trunk with water), but also to other factors including those just mentioned.

It is obvious that defoliation would cause less disturbance to the hydrostatic system within the plant than decapitation. In illustration, two small walnut trees (*Juglans regia*), about 1.5 meters in height and 3 cm. in diameter at the base, were defoliated on July 27, 1927, in order to diminish the tensions on the water-columns. Each tree had a leaf-surface of about 125 sq. cm. before the leaves were removed. At the time of defoliation there was a rather high deficit of water within the plant, as is evidenced by the fact that portions of the stem absorbed 20 per cent of their weight of water in 2 days. A manometer was attached to the stump of a side branch in each of the defoliated plants, and one of the plants was profusely irrigated, and the irrigation continued during the period of the experiment. The other plant, growing in a rather dry soil, was unirrigated. With the exception of irrigation, the other external factors were approximately the same. The experiment ran from July 27 to August 31, 1927, during which time daily records were kept. In the irrigated tree the manometric variations showed suctions ranging from 0 to 29 mm. Hg., but usually about 4 mm.; the suctions being least on cloudy or foggy cool days, and greatest on warm bright days. The same is true between morning and midday, and between midday and evening. In the unirrigated plant the suctions ranged from 0 to 42 mm. Hg., usually about 35 mm., the suctions varying with the temperature changes. It is evident that the amount of moisture about the roots of these plants affects to a certain degree the suctions manifested by manometers attached to branches, suctions being less in irrigated plants.

It is interesting to note that similar suctions obtain in certain plants which have become defoliated through natural causes. *Jatropha*, a woody shrub growing in the desert near Tucson, Arizona, sheds all of its leaves soon after the end of the winter rainy season. This desert plant, which always shows a marked water deficit, never shows an exudation-pressure in manometers, even if profusely watered, although suction-pressures may be somewhat lessened by irrigation. Suction-pressures were found both under natural desert conditions and when plants were transported to a greenhouse, where transpiration was reduced to a minimum.

The results of defoliation on larger trees have already been published by MacDougal.¹ It was shown that no extensive exudation-pressures are possible in defoliated Monterey pines. Such pressures as are exhibited he attributes to the secretion and hydration of resin.

All of our experiments on exudation-pressures thus far considered have been on trees, either large or small, and on woody shrubs. We have also investigated exudation-pressures of herbaceous plants. The herbaceous plants studies include the sunflower (*Helianthus*), *Malva borealis*, and *Encelia farinosa*. *Encelia* is a much more woody plant than *Malva* or *Helianthus*. The observations on these three species were made as they were growing under the natural desert conditions at Tucson, Arizona, and during April and May, following the cessation of the winter rains. Intact or decapitated individuals of these three species show no positive pressures, either in stems or roots, when the plants are growing under the arid conditions characteristic of the region at this season of the year. When these same plants, which have been showing suctions only, are profusely irrigated they show positive pressures in the manometers attached to the stem or to the root. The difference between the behavior of these herbaceous plants and the woody plants previously described undoubtedly lies in the extent of the endodermal mechanism in the two types of plants. As mentioned on page 86, the endodermis ensheathes the greater portion of the stele in root-systems of herbaceous plants. Woody plants, on the other hand, have but a relatively small proportion of the stelar tissues in their roots surrounded by an endodermis.

It is obvious that another factor affecting suctions and pressures is the structure of the xylem. Quantitative studies on the water-conducting capacity of plants have been made by several investigators since Farmer² and his students recorded that the "specific conductivity" of the wood varies from species to species, from individual to individual of the same species, and from branch to branch of the same tree. Farmer's students, Holmes^{3, 4} and Rivett,⁵ have endeavored to determine the causes underlying this variation, and have come to the conclusion that variations in conductivity are due to the resistance offered by the differences in structure of the wood. The chief factors which they take into consideration are the size, the proportion, and

¹ MacDougal, D. T. The hydrostatic system of trees. Carnegie Inst. Wash. Pub. No. 373. 1926.

² Farmer, J. B. On the quantitative differences in the water-conductivity of the wood in trees and shrubs. Part II. The deciduous plants. Proc. Roy. Soc. London, B 90: 232-250. 1918.

³ Holmes, M. G. A study in the anatomy of hazel-wood with reference to conductivity of water. Ann. Bot., 32: 553-567. 1918.

⁴ ———. Observations on the anatomy of ash-wood with reference to water-conductivity. Ann. Bot., 33: 255-264. 1919.

⁵ Rivett, Maud F. The anatomy of *Rhododendron ponticum* L. and *Hex aquifolium* L. in reference to specific conductivity. Ann. Bot., 34: 525-550. 1920.

the distribution of the water-conducting elements in the wood. Farmer finds that the efficiency of the wood of "evergreen" trees in conducting water is relatively low as compared with that of deciduous trees. Huber¹ states that the amount of water which will filter through equal cross-sectional areas under equal pressure or transpirational pull is about one-fourth greater for deciduous than for evergreen trees. In the two evergreens studied by Miss Rivett (*Ilex aquifolium* and *Rhododendron ponticum*) the differences in specific conductivity are ascribed to differences in percentage of wood occupied by cavities. This is also held to be true of two deciduous plants studied by Holmes, the ash and the hazel. The greater specific conductivity of the deciduous plants over that of the two evergreens is explained by the greater length of vessels in the deciduous plants and the differences in the character of the perforations in walls of the conducting elements in the two types. Aitken² finds that *Pinus pinaster* has a wood of low water-conducting power, slightly higher than that found by Farmer for *Pinus sylvestris*, whereas *Leucadendron argenteum* has wood with a high conducting power for an evergreen.

After studying the specific conductivity of both evergreen and deciduous forms, Inamdar and Shrivastava³ conclude that the average diameter of the vessels varies in the same direction as the specific conductivity, and is correlated with the demands made by transpiration. These authors hold, however, that the diameter of the vessels is only one of the contributing factors in specific conductivity, since there is no quantitative proportionality maintained between the diameter of the vessels and specific conductivity. They conclude that the length of the vessels and the proportion of conducting elements to the total volume of the wood are also important factors. Inamdar and Shrivastava agree with Farmer, that the characteristic differences between the specific conductivity of saplings and of adult trees are correlated with differences in the diameters of the vessels, saplings possessing vessels of a smaller diameter than those in adult trees.

In the beech Huber^{4 5} finds that the vessels average 26.4 microns in diameter and occupy 35 per cent of the cross-sectional area of the stem. In the spring wood of the pine the tracheids have an average

¹ Huber, B. Beiträge zur Kenntnis der Wasserbewegung in der Pflanze. II. Die Strömungsgeschwindigkeit und die Grösse der Widerstände in den Leitbahnen. Ber. Deutsch. Bot. Ges., 42: 27-32. 1924.

² Aitken, R. D. The water relations of the pine (*Pinus pinaster*) and the silver tree (*Leucadendron argenteum*). Trans. Roy. Soc. S. Africa, 10: 5-19. 1921.

³ Inamdar, R. S., and A. L. Shrivastava. The relation between the specific conductivity and the structure of the wood elements in the tropical plants. Jour. Indian Bot. Soc., 4: 304-306. 1925.

⁴ Huber, B. Die physiologische Leitungsfähigkeit des Wasserleitungssystems der Pflanze. Ber. Deutsch. Bot. Ges., 43: 410-418. 1925.

⁵ ———. Oekologische Probleme der Baumkrone. Planta, 2: 476-488. 1926.

diameter of 17.8 microns, and 8.6 microns in summer wood, and occupy 36 per cent of the cross-sectional area. The wood of the beech shows a conductivity 10 times as great as the spring wood of the pine and 12 times as great as that of the summer wood in the pine. He points out that the conductivity is much less than can be ascribed solely to the friction offered to the moving water-columns by the side walls of the conducting tracts, and he concludes that differences in conductivity between the wood of beech and that of the pine can be ascribed to resistance to passage offered by the end-walls.

Our experiments on the injection of dyes into gas-filled tracheids and vessels of intact trees (page 46) furnish an index of the efficiency of conduction (specific conductivity) in the trees with which we have experimented. In the Monterey pine, whose tracheids are 0.62 to 4.15 mm. long, dyes can be forced but slowly into gas-filled elements. In the live oak (*Quercus agrifolia*), whose vessels are 33 to 35 cm. long, dyes are forced into gas-filled elements with great ease. Dyes can also be readily injected into the gas-filled vessels of *Salix lasiolepis*, which are 7 to 10 cm. long, but not nearly so readily as into those of the oak. Injection experiments with the alder (*Alnus oregona*), with vessels 7 to 8 cm. long, show only a relatively slow movement from vessel to vessel. It is evident from the foregoing that length of the conducting elements is one of the major factors in specific conductivity. That this is not the sole factor is shown by differences in speed of penetration of gas-filled vessels in the willow and the alder, which have vessels of approximately the same length and breadth.

Our experiments showing that certain portions of an annual layer may be occupied exclusively by water, and that other portions may contain only gas, appear to have an important bearing on the specific conductivity of xylem. When vessels, large or small, short or long, are filled with gas their conductivity is nil. From this it would appear that the experiments of Farmer and others give only a true picture of the specific conductivity of the water-filled portion of the wood with which they experimented. If the wood were entirely filled with water the same piece would give an entirely different specific conductivity. For example, we would expect, under midsummer conditions, that the specific conductivity of the willow would be increased three- or fourfold if the enclosed gases were removed from the wood. From this it follows that this neglected factor of gas-filled vessels and tracheids is of much greater importance in determining conduction through the stem than the number and position of conducting elements and their length, breadth, and structure. We also hold that the seasonal variation in specific conductivity described by Inamdar and Shrivastava¹ is the direct result of changes in the proportion of

¹Inamdar, R. S., and A. L. Shrivastava. Seasonal variation in specific conductivity in tropical plants with reference to leaf-fall. Bot. Gaz., 83: 24-47. 1927.

water-filled and gas-filled conducting elements, and not necessarily correlated, as they state, with the varying demands made on the water-supply by the transpiring leaves.

Changes in the proportion of gas-filled and water-filled vessels and tracheids, which have just been mentioned, are in large part dependent upon the nature of the communications between adjoining vessels and tracheids. Dixon¹ states that, when a bubble forms within a vessel or tracheid, the water around the bubble is drawn away by the tension, and the surface of the bubble comes to rest against the wall of the element in which it has developed. Further enlargement of the bubble is only possible in a longitudinal direction, although the enlargement may continue until it completely fills the lumen of the element. He holds that further enlargement is impossible, because the surface of the bubble is restrained in all sides by the wall of the element, even through the pits which "oppose an impermeable barrier to undissolved gases."

This contention, that the membranes of all bordered pits are septa entirely impervious to undissolved gases, is denied by Bailey.² Bailey bases his contention both on cytological and experimental study of the perforations in closing membranes of bordered pits of conifers. He has demonstrated the actual passage of liquids with minute particles in suspension, as India ink, from tracheid to tracheid through the openings in the closing membranes. Since Bailey's contention has been denied in certain quarters, we have repeated his experiments, using living material of the Monterey pine, confirming and extending them. We first used Chinese ink, finely ground in a mortar in distilled water, and centrifuged at 3,800 revolutions per minute in order to remove all but the very smallest particles. This suspension of minute carbon particles was found to travel, under the pull of a suction-pump, for a distance of 20 to 30 mm. up a freshly cut pine stem, a distance many times the length of a tracheid. The same results were obtained when the severed top of a tree was stepped into a suspension of the centrifuged ink.

These experiments were repeated, using, instead of Chinese ink, a suspension of a finely ground graphite obtained from "Aquadag," a commercial preparation of graphite used for lubrication. This substance, as marketed, contains a small amount of ammonia. In order to remove the ammonia the suspension of "Aquadag" was allowed to evaporate, leaving a hard mass of graphite, from which a suspension was made and centrifuged. The resulting suspension contained only the finest particles of the original suspension. When graphite is suspended in water, graphitic acid may be formed, which in the presence

¹ Dixon, H. H. *Transpiration and the ascent of sap in plants*, p. 91. 1914.

² Bailey, I. W. The structure of the bordered pits of conifers and its bearing upon the tension hypothesis of the ascent of sap in plants. *Bot. Gaz.*, 62: 133-142. 1916.

of ammonia may form a salt, or the free ammonia may be absorbed by the graphite. Either of these compounds may yield free ammonia under the conditions of the experiment. Ammonia if present might account for a certain blackening of the walls, but no appreciable amounts of free ammonia were found in the suspension by the ordinary qualitative tests. The suspension of graphite gave the same results as the suspension of Chinese ink. It should be noted that in determining the passage of either Chinese ink or graphite from tracheid to tracheid, we have depended upon observing the actual presence of the particles, and not upon the discoloration of the cell-walls.

Experiments on the walnut and the willow, similar to those described above, show that there is not a passage of even the finest carbon particles beyond the length of a vessel. However, our results on the transmission of suctions and pressures from one gas-filled element to another (page 41) show that suctions and pressures are readily transmitted considerable distances within a very short time. These results seem to justify the assumption that perforations of lesser diameter than can be passed by carbon particles or the sub-microscopical particles in a colloidal oxyferric chloride suspension, offer communication from vessel to vessel. In the case of water-filled vessels these openings are so small as to present great resistance to the movement of water from vessel to vessel. Renner¹ has suggested that these exceedingly minute perforations in the closing membranes of pits, through which the tensile water-columns are continuous, are perforations formerly occupied by *plasmadesmen* when the vessels contained protoplasts.

It is the presence of these microscopic or ultramicroscopic perforations in closing membranes of bordered pits of vessels and tracheids which permits the movement of gas from a gas-filled element into an adjoining water-filled element. This movement may be from vessel to vessel that are in lateral contact: it may possibly take place even more readily from vessel to vessel that are in vertical alignment with one another. Thus, gas originating in a specific element may not only eliminate that element as one which conducts water, but may also eliminate adjoining elements from the water-conducting system.

Bailey has computed the force necessary to cause a movement of gas from a gas-filled to a water-filled tracheid. The largest perforations in closing membranes of bordered pits of *Larix* are about 3 microns in diameter, and he estimates that a pull of 1 atmosphere is sufficient to overcome the surface-tension of the water in the perforation. In the case of the smallest perforations of *Larix*, some 0.5 micron in diameter, a pull of 5.8 atmospheres is necessary. Undoubt-

¹ Renner, O. Die Porenweite der Zellhäute in ihrer Beziehung zum Saftsteigen. Ber. Deutsch. Bot. Ges., 43: 207-211. 1925.

edly much higher pulls are necessary to cause gases to pass through the ultramicroscopic perforations in closing membranes of the willow and the walnut, but we believe that a cohesive transpirational pull of a hundred atmospheres or so would be sufficient to cause the entrance of gas into water-filled vessels in these trees.

THE RÔLE OF LIVING CELLS IN SAP-FLOW

The earlier contentions as to the necessity of living cells for the ascent of water, as presented by Godlewski,¹ and supported by Westermaier² and Janse,³ were shown to be inadequate by Strasburger.^{4, 5} Strasburger, in a series of elaborate experiments, killed long portions of stems by various means, including poisons, and showed that the killing of the living cells in the stem did not materially affect sap-flow. Although several authors have refused to accept Strasburger's results, it has, however, been shown by one of us⁶ that when a portion 5 to 10 cm. long in the stem of *Cyperus* was killed with certain poisons, including picric acid, sufficient quantities of water ascended to supply the transpiration needs for a long period, and long enough to allow the development of new branches.

When living willow and walnut trees are stepped in poisonous solutions, as picric acid, there is the same general distribution of the poison as when the trees are stepped in acid fuchsin, except for a more general diffusion of the picric acid. These experiments of Strasburger and of Ewart were repeated by sawing off a willow tree 11 cm. in diameter and about 8 meters high, lowering the trunk into a concentrated aqueous solution of picric acid, and allowing it to stand and transpire in the solution for four days. A section was then sawn off for examination as to the distribution of the poison. The tree was then stepped into acid fuchsin. In four days the tree absorbed 10 liters of picric acid solution, and about the same amount of acid fuchsin in the succeeding four days. Both the picric acid solution and the acid fuchsin were drawn up the tree for a distance of about 6 meters. In all layers of wood the distribution of the poison was nearly the same as has been described (page 35) for trees stepped in acid fuchsin only, except that the lateral diffusion was greater, being very diffuse in the youngest four layers. The distribution of the acid fuchsin in poisoned stems is exactly like that in unpoisoned wood, except that a lateral diffusion of the dye occurs in the poisoned medullary rays and wood parenchyma. On one side of the tree the lateral diffusion of the dye was more extensive than on the other. The experiment was repeated with a small walnut tree 10 cm. in

¹ Godlewski, E. Zur Theorie der Wasserbewegung in den Pflanzen. Jahrb. Wiss. Bot., 15: 569-630. 1884.

² Westermaier, M. Untersuchungen über die Bedeutung todter Röhren und lebender Zellen für die Wasserbewegung. Sitzber. Preuss. Akad. Wiss., Jahrgang 1884: 1105-1117. 1884.

³ Janse, J. M. Die Mitwirkung der Markstrahlen bei der Wasserbewegung im Holze. Jahrb. Wiss. Bot., 18: 1-69. 1887.

⁴ Strasburger, E. Ueber den Bau und Verrichtungen der Leitungsbahnen in den Pflanzen. Histol. Beitr., 3: 1-1,000. 1891.

⁵ ———. Ueber das Saftsteigen. *Ibid.*, 5: 1-94. 1893.

⁶ Overton, J. B. Studies on the relation of the living cells to transpiration and sap-flow in *Cyperus*. Bot. Gaz., 51: 28-63, 102-120. 1911.

diameter and 6 meters high. Both the picric acid and the acid fuchsin were drawn up 4 to 5 meters. As in the case of the willow, the course of conduction was identical with that in trees stepped in acid fuchsin only, except that both the picric acid and the acid fuchsin showed a certain amount of lateral diffusion both in the medullary rays and in the wood parenchyma.

Ewart's¹ hypothesis that the living cells in the stems of trees in some way restore the conditions necessary for the ascent of water and decrease the resistance to flow, and the contention of Ursprung² that the function of the living cells is to keep the vessels in a proper state of conductivity seem not to be supported by our experiments, except in so far as living cells affect the osmotic concentration of the transpiration stream.

In contrast with the conclusions of these skilled investigators, who have used admissible methods and dealt directly with facts experimentally obtained, are the assertions of Bose³ as to the pulsatory movement of sap in living cells, especially in cortical tissues. These assertions, which are seriously presented in scientific journals and widely exploited by writers with no scientific training, are not supported by any recorded facts as to the path, rate, or forces which move solutions upward through tall stems. Minute rhythmic variations in the thickness of stems are assumed to be connected with the propulsion of sap from cortical cell to cortical cell, and no facts are offered to show that such movement does take place. Molisch,⁴ whose experiments in this field have been chiefly confined to a study of exudation-pressures, has recently repeated the measurements of the rhythmic action of stems with Bose's apparatus, but with an equal lack of identification of these phenomena with actual movement of liquid in the stem. The pulsatory movement of sap claimed by Bose and by Molisch would imply transfer through two to four hundred living cells per second. Benedict,⁵ after measuring the sap-flow in ten species of trees, concludes that the actual rate of flow of sap in these trees is from eight thousand to thirty thousand times as great as would be possible under the conditions and by the path postulated by Bose. In further illustration of the fantastic disregard of fact which characterizes Bose's papers on this subject, it may be cited that he attributes the origin of the pulsations in the hydrostatic system to the stimulation of the roots by friction from soil particles with which they are in contact.

¹ Ewart, A. J. The ascent of water in trees. *Phil. Trans. Roy. Soc. London*, B 199: 341-392. 1908.

² Ursprung, A. Die Beteiligung lebender Zellen am Saftsteigen. *Jahrb. Wiss. Bot.*, 42: 503-544. 1905.

³ Bose, J. C. The physiology of the ascent of sap. London, 1923.

⁴ Molisch, H. The movement of sap in plants. *Nature*, 121: 168-169. 1928.

⁵ Benedict, H. M. Application of Bose's theory of sap-rise to ten species of trees. *Am. Jour. Bot.*, 14: 623. 1927.

CONCLUSIONS

The results of the experiments described in the preceding pages make it abundantly clear that contributions to the subject of the ascent of sap in trees, or to movements of gases and liquids, are to be made on the basis of the results of intensive study of types of stems in which the interlocking action of the hydrostatic and pneumatic systems may be made out. Localization of the paths of greatest movement of solutions, determination of lateral and longitudinal movement between vessels, and communication of the pneumatic system with the atmosphere may be accomplished only by accurate visualization of the minuter anatomy of the vessels, tracheids, rays, cambium, and tissues external to the cambium. The fact that the movement of sap in *Alnus* has been found to be through the spring wood, that in *Salix* through the late summer wood, and that in *Juglans* through the early spring and late summer wood should serve as a warning of the futility of attempts to correlate too exactly phenomena from different types of trees. Identity of action in *Salix*, *Populus*, and *Juglans* is by no means as close as might be expected from their morphological similarities. The oak tree studied is a species characterized by a great capacity for forming callus, a thick spongy bark, including much living tissue and a chlorophyllose outermost layer, and a wood with much less hardness than that usually attributed to the oaks. Each suite of leaves is retained throughout the cooler season, and until the beginning of the season of active growth, a feature which results in its familiar name of "live oak." The mechanism of its gaseous and water systems will probably be found to vary in many features from those of the larger deciduous oaks of eastern America and Europe.

The possibility of such specialization may be profitably taken into account in any researches upon "root-pressure," and in attempts to trace the path and define the forces by which sugars and other complex compounds are translocated from one tissue to another, especially from the foliar organs to all parts of the stem and root system.

A comprehensive picture of the hydrostatic and pneumatic systems of the pine, of the willow, of the walnut, and of the oak, upon which our observations have been principally carried out, would include the following features:

1. A cohesive meshwork of sap occupying portions of all untylosed annual layers of these trees.

2. The cohesive columns of water occupying the tracheids and vessels are in a state of tension set up by evaporation from the exposed walls of cells adjoining intercellular spaces of leaves.

3. Dendrographic studies made show that the pull set up by water-loss from such surfaces causes daily variations in size of intact stems and trunks, owing to an increase and decrease of the tension.

4. Our experiments indirectly confirm the contention of several investigators that the tensions set up by the transpiring leaves may amount to as much as two hundred atmospheres.

5. The tension set up by the transpirational pull exerted by leaves of the pine is directly transferred to the layers with which the leaves are connected. This results in a more rapid movement through the second, third, and fourth annual layers next the cambium, and a very much slower movement in the older layers. We are uncertain concerning movement through the outermost annual layer.

6. The rate of upward movement in the dicotyledonous trees that we have studied is more nearly equal in the various annual layers, although there is some irregularity.

7. In the pine there may be a certain amount of radial mass movement of the transpiration stream, through the ray tracheids, from one annual layer to that external to it. There is probably a further mass movement between such layers by the same method as obtains in dicotyledons.

8. There is, under midsummer conditions, no radial mass movement of the transpiration stream from one annual layer to that external to it in the dicotyledonous trees that we have studied. Movement of the transpiration stream from one layer to the layer external to it is only in the vertical direction and in the region where one layer caps and ensheathes the other.

9. Movement of liquids within any given annual layer is many times more rapid in the longitudinal than in the radial or the tangential direction. The greater speed of longitudinal movement may be correlated with the smaller number of partitions to be traversed.

10. The pine has perforations in the closing membranes of its bordered pits of sufficient magnitude to permit the passage of suspended carbon particles from one tracheid to another. The dicotyledonous trees examined do not have perforations of this magnitude. Although such perforations as are found in the pine undoubtedly facilitate mass movement of liquids or gases from one conducting element to another, the slower mass movement through wood of pine, as compared with that of dicotyledons, may be ascribed to the greater number of partitions to be traversed.

11. Irregularly arranged tracts of tracheids in the pine and definite vertical zones of vessels within each untylosed annual layer of the willow, the walnut, and the alder are occupied by gases during the summer months.

12. The proportions of the component gases in the trunks of the trees examined are widely different from those of the atmosphere.

13. Pressures and suctions on the gaseous system within the trunk are readily transmitted vertically for distances many times the length of the vessels. Tangential transmission of suctions and pressures is at a very slow rate, and is even slower in a radial direction.

14. The internal gaseous system of the tree is, however, in communication with the atmosphere radially through the cambium and vertically through the ends of the branches. Differences of as much as half an atmosphere may cause a flow of gases from the outside air into the trunk.

15. The relative volumes of the hydrostatic and pneumatic systems within the tree are subject to variation during the course of the season. Specific conducting elements may at one time be partially or wholly filled with gas, and at another time entirely filled with water.

16. Tensions in the pneumatic system may vary from something less than half an atmosphere to not more than one or two atmospheres. Tensions of the hydrostatic system may vary from a compression or positive pressure to a suction or pull of one to two hundred atmospheres.

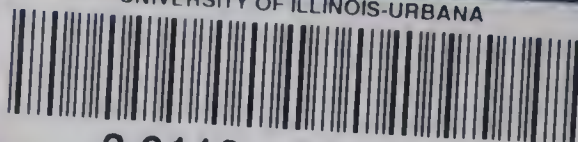
17. Varying pressures of the internal pneumatic system exercise only a minor influence on the tension in the hydrostatic system.

18. The "root-pressures" found, under certain conditions, in herbaceous plants, with more or less intact endodermal mechanisms, have not been found in the coniferous or dicotyledonous trees with which we have worked, nor in woody shrubs.

19. No information has been obtained by which root-pressure may be identified with or separated from exudation-pressures in cut stems, branches, and roots, or with the secreting action of nectaries and glands. Exudation-pressures originating in resin-cells of roots, trunks, and branches of conifers, and in laticiferous elements of other plants, have been wrongly classed as root-pressure.

20. Our experiments show that the living cells of the stem have no active rôle in sap-flow other than their effect upon the osmotic concentration of the transpiration stream.

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